INVESTIGATIONS OF MONOSOMICS IN OATS, *Avena byzantina* C. Koch.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Monosomics and their derivatives in Triticum aestivum and in Nicotiana tabacum have been effectively used (1) to identify and locate genes, (2) to transfer genes from one genetic background to another, and (3) to study gene dosage relationships. It is the genetic redundancy of polyploids which enables them to survive, usually with little change, the loss of a whole or a part of a chromosome from their genetic complement, whereas monosomes of diploids are rare. Hence a given polyploid species can produce aneuploids by either natural or induced changes, which are very useful in cytogenetic investigations.

Such opportunities have led cytogeneticists to induce, identify, and establish monosomics and their derivatives in cultivated hexaploid oats, Avena sativa L. The literature shows a few reports of spontaneous chromosome losses accompanied by certain characters in hexaploid oats. Recently, ionizing radiations and mutagenic chemicals have been used to induce aneuploids at higher frequencies.

Two major obstacles in establishing and using monosomics of hexaploid oats are the lack of morphological distinction between many chromosomes and that of unclear genome relationships. Improved techniques of karyotype analysis along with cytogenetic experiments have helped considerably in identifying critical chromosomes, but they are not adequate. It is anticipated that elucidation of the relationships of various pairing control mechanisms in genomes of hexaploids,

tetraploids, diploids, and even related genera, may shed light on the real progenitors of the B, C, and D genomes of Avena species. Once the genome relationship is clearly understood and the monosomic lines for all the chromosomes are developed, it will be much less difficult to identify and assign the chromosomes to their proper genomes.

The present work involves (a) the screening of monosomic lines from 84 lines induced for resistance to Helminthosporium victoriae M. and M. by Co^{60} gamma-irradiation and certain mutagenic chemicals, (b) the study of breeding behavior of selfed monosomics in all lines, (c) identification of chromosomes in all monosomic lines based on karyotype analysis, (d) cytological behavior of disomics, monosomics, nullisomics, and any other type of selfed monosomics progeny, (e) association of characters (morphological and cytological) with critical chromosomes in different monosomic lines, in Avena byzantina C. Koch.

Since all previous efforts to establish monosomics and their derivatives have been confined to common oats, Avena sativa L., these studies propose to investigate the above mentioned phenomena in red oats, Avena byzantina, another allohexaploid species of economic importance. The objectives are not only of applied importance but are sound theoretically as well. Thus if monosomic lines are separately developed both in A. sativa and A. byzantina, identical chromosomes in the two species can be compared with regard to their gene contents and pairing relationships; their evolutionary relationships may be also revealed.

During the process of isolation, identification and characterization of monosomic lines, experiments were in progress to test whether

any of these lines and five monosomes obtained from Ames, Iowa, involved the critical chromosome carrying the vb locus, responsible for reaction to H. victoriae. The inheritance of Vb locus seems to be monogenic as reported by various workers (15, 40, 49). Susceptibility to the fungus or to its toxin is dominant (Vb) over resistance (vb), and earlier reports indicate that this locus, or loci very closely linked to it, controls the resistance to a few races of crown rust caused by Puccinia coronata Cda. f. sp. avenae Fr. and Led. an obligate parasite. Investigations of possible allelic complementation at the Vb locus are being planned for future studies.

LITERATURE REVIEW

The species of Avena have been classified according to their floral structure and chromosome number as diploid (n=7), tetraploid, (n=14), and hexaploid (n=21). The genomic formulae AA, AABB, and AABBCC were proposed respectively for the above classes by Nishiyama (50, 53, 54). Rajhathy and Morrison (66, 67) based on karyotype and pairing studies of Avena species, proposed that the hexaploid oats genomes should be AACCDD. The relationships among the species of Avena are not as clear as those among species of Triticum, in which the immediate phylogeny of the hexaploid species is known with certainty. One part of the general problem of speciation and phylogeny in Avena, however, is very closely involved with deficiency. This is the relationship between A. sativa or A. byzantina with A. fatua, which is discussed below.

The nature and origin of fatuoid plants has been a most important and controversial subject of hexaploid oats cytogenetics since it was first reported by Buckman (5). Three hypotheses have been forwarded to explain the origin of fatuoids namely, mutation, natural crossing, and chromosomal aberration. Since this project has been limited to chromosomal deficiencies, only the third hypothesis will be discussed here.

Goulden (18) discovered a dwarf oat plant in the progeny of plants heterozygous for false wild characters. These dwarfs, about one half the normal plant height, were completely sterile and always possessed false wild characters. When dwarfs occurred in families from heterozygous plants for the false wild characters, the cultivated class was practically eliminated and the remainder consisted of false wild dwarfs and intermediate normals in a 1:1 ratio. In the cytological examination of pollen mother cells (PMCs) Goulden (18) noticed complete lack of coordination among the chromosomes at the heterotypic division, and no definite homotypic division. Very few normal appearing pollen grains were formed. He summed up the results this way: "There is evidently in the case of oat dwarfs an intimate correlation between dwarfing, the false wild characters, cytological irregularities, and complete sterility." Unfortunately he did not determine the chromosome number of dwarfs or heterozygous normals.

Following Goulden's discovery, Huskins (23, 24) made it clear that fatuoids arise from normal oats neither by natural crossing between A. sativa and A. fatua L. nor by gene mutation, but by chromosome aberration. This conclusion was partially influenced by the Winge's (84) hypothesis that the allopolyploid origin of Triticum aestivum could account for chromosomal association between nonhomologous or homologous chromosomes.

Huskins (23, 24) recognized three types of heterozygous fatuoids and designated them as A, B, and C series (or α, β, γ, as he renamed them in 1946). Heterozygous fatuoids in the α series gave normals, heterozygous fatuoids, and homozygous fatuoids, all of equal vigor, in a ratio of 1:2:1. All had 42 chromosomes, but the normals

showed 21 II, heterozygous fatuoids 19 II + III + I, and homozygous fatuoids 19 + IV. This was attributed to a very small deficiency or duplication of fatuoid factors. Heterozygous fatuoids in the β series gave normals and heterozygous fatuoids in equal frequencies with few homozygous fatuoids or none at all. The chromosome numbers for the above three classes were 42, 41, and 40, respectively. Homozygous fatuoids with 40-chromosomes had very irregular meiosis and were completely sterile. In the γ series normals had 42-chromosomes, heterozygous fatuoids 43-(20 II + III or 21 II + I) chromosomes, and homozygous fatuoids 44-(22 II or 20 II + IV) chromosomes. The homozygous fatuoids in the γ series were dwarf and sterile. Their meiotic divisions were not very irregular, but the contents of their pollen grains degenerated during the later stages of their development. Thus Huskins (23, 24) was the first to associate irregular meiosis and expression of fatuoid character with the loss or duplication of a particular chromosome.

Because of the great interest in the fatuoid character, Nishiyama (51) obtained Huskins' (23, 24) material by way of Prof. Kihara. He modified the classification of Huskins (23, 24) and renamed the fatuoid series as Series I and II. Series I corresponded to α series of Huskins, whereas Series II was divided into types a, b, and c, with type a and type c the same as series β and γ of Huskins and type b, a modified β series of Huskins. In the modified β series (type b), both normal and deficient gametes performed equally well.

Nishiyama (51) picked up two heterozygous fatuoids from the α series of Huskins which segregated dwarf homozygous fatuoids, heterozygous fatuoids and normals in a ratio of 1:1.5:0.1. The dwarf

homozygous fatuoids had 40-chromosomes and showed extremely irregular meiosis. Therefore they were completely sterile. A few homozygous fatuoids with low fertility were found, and presumably they had 20 pairs plus a telocentric for the long arm. Heterozygous fatuoids had 20 II + 1 I, and the seed set on these plants varied from 27 to 94 per cent depending upon the environment. Nishiyama (52) pointed out that fatuoid characters appeared in nullisomic-c because the short arm of the c-chromosome carried a factor or closely linked factors for the cultivated type grain character, and asynapsis because the long arm carried a synaptic factor that was necessary for the normal pairing of chromosomes. Thus Nishiyama (52) described the irregular meiosis identified by Huskins (23, 24) as being due to asynapsis.

From later investigations Huskins and Hearne (26) and Huskins et al. (27) concluded that the long arm of the c-chromosome of fatuoids had genes for normal grain type and the short arm a gene controlling normal chromosome pairing. Nishiyama (52) reported the opposite results.

Soost (81) reviewed the literature on asynapsis and classified it into five groups: (a) asynapsis in species hybrids, (b) asynapsis caused by the loss of a chromosome pair, (c) asynapsis in apomictic organisms, (d) asynapsis induced by external cause, (e) asynapsis due to action of a gene or genes. Each of the above groups were characterized by unpaired chromosomes at the first meiotic metaphase even though the cause in each group may have been quite different. Where asynapsis was caused by the loss of a chromosome pair, the return of this pair restored pairing (26, 27, 51, 52). When unfavorable environmental conditions, such as extreme temperatures (13, 79) and

deficient water (60) were removed, pairing also returned to normal.

Maize provides the most thoroughly investigated example, namely that of asynapsis caused by a recessive gene located in the chromosome 1 (2, 3). Recessive genes causing asynapsis are common in other species. They are known in Crepis (69), Datura (4), Lycopersicon (81), Pisum (33), Secale (60), Sorghum (34, 62), and Triticum (39).

On the other hand, systems are also known where absence of a gene or genes result in extreme pairing which in turn results in multivalent formation. It is evident from the reports of a number of workers (29, 32, 70, 71) that in hexaploid wheat, Triticum aestivum, completely homologous pairing is conditioned by the long arm of the chromosome V which is in the B genome. In its absence homoeologous pairing occurred, resulting in multivalent formation. Thus the diploid-like meiotic behavior of hexaploid wheat, T. aestivum, is under strict genotypic control. A similar genetic system analogous to that controlling diploidization in wheat has been suggested in tetraploid cotton by Kimber (30).

Another important character to be associated with loss of a whole sub-median chromosome in A. sativa, albinism, was first reported by Philp (58). The univalent transmission rate was found to be 10.1 per cent. He designated this chromosome as V; its loss in somatic tissue was easily recognized. Based on these and some additional observations, Philp (58) suggested that polymeric genes (V genes) are responsible for chlorophyll production and that at least two or probably three loci are present on different chromosomes in common oats, because of its hexaploid nature. Further, he pointed out that this V-chromosome was definitely different from the c-chromosome reported by Nishiyama (51, 52).

McGinnis and Taylor (44) associated a gene for chlorophyll production with the shortest subterminal chromosome in A. sativa. Disomic and monosomic plants for the critical chromosome were green, whereas nullisomics were albino. The univalent chromosome was included only in 16.8 per cent of the pollen grains and had little effect on the viability of deficient pollen grain. Consequently the nullisomic frequency was reported to be quite high (69.2%). That the gene controlling chlorophyll synthesis was on the long arm of the critical chromosome was later reported by McGinnis et al. (45).

Investigating the problem further, McGinnis and Andrews (43) reported a second chromosome involved in chlorophyll production. This chromosome was found to be the shortest submedian; from the presence of a telocentric in one of the albino seedlings, they concluded that the gene for the chlorophyll production was associated with the long arm of the above chromosome. This may be the same chromosome as that reported by Philp (58).

Philp (59) reported a hybrid from a cross between A. sativa and A. fatua which segregated for broad and narrow-leaved seedlings in a ratio of 1:2. Cytological examination revealed broad-leaved plants to have 41 chromosomes whereas narrow-leaved plants had only 40 chromosomes. He designated this chromosome as L and stated that it was different from the V and c-chromosomes. The univalent transmission rate was 6.0 per cent, with close agreement between the observed and expected frequencies.

Nishiyama (55) studied the steriloid mutants (intermediate base type), which were found in the F_3 offspring of triploid hybrids between A. barbata (2n=28, articulated base type) and A. strigosa (2n=14,

solidified base type). Monosomic plants (2n-1=27) were characterized by slender culms, weak spreading of culms, and less vigor. Kernels developed normally and germinated well at a rate of 88.49 per cent. The univalent had a subterminal centromere as observed at the first and second telophase and the transmission rate of 43.10 per cent.

Ramage and Suneson (68) isolated a highly self sterile nullisomic line in A. byzantina in which the sterility was attributed to the missing pair of chromosomes. Reductions in plant height, tiller, and floret production were found to be 45.0, 50.0, and 65.0 per cent, respectively. All pollen grains were stained with iodine solution, but 95.0 per cent were found to be shrivelled in varying degrees, and selfing resulted in only 10.0 per cent seed set. They pointed out that the missing chromosome was not one of the three satellited pairs or the shortest pair with a subterminal centromere and emphasized the point that unlike nullisomic fatuoids described by Huskins (23, 24), this nullisomic was relatively stable through meiotic divisions and had sufficient fertility to be used in breeding programs. Frequencies of disomics, monosomics, and nullisomics were not reported.

Costa-Rodrigues (12) used X-rays to induce monosomics in A. sativa and obtained 7.2 per cent monosomic plants with an irradiation treatment of 300 roentgens. He examined progenies of five monosomic lines and observed a range of 0 to 46.0 per cent nullisomic plants from the different lines. He pointed out that most of the monosomics involved small chromosomes, but his data were not sufficient to support the above statement.

McGinnis (42) found three monosomics and two completely sterile nullisomics among 24 aneuploids studied in A. sativa. The fertility of aneuploids ranged from complete sterility to 30.0 per cent seed set. Monosomics for chromosomes 14, 20, and 21 (renamed 21, 10, 15, respectively, by Rajhathy, 1963), were most fertile with up to 30.0 per cent seed set on some plants. Plants nullisomic for chromosome 14 exhibited a higher fertility than their parent monosomics.

Out of 3,453 seedlings, 53 aneuploid seedlings were isolated by Hacker and Riley (20) from natural populations of A. sativa. The percentage of aneuploids varied from 1.2 to 1.9 per cent. Monosomics occurred with a greater frequency (40 seedlings out of 3,453) than other classes, but nullisomics, trisomics, and plants with one or two telocentric chromosomes were also recorded.

Andrews and McGinnis (1) used X-rays, sonic vibrations, and mutagenic chemicals (Myleran, 8-ethoxycaffeine) to induce aneuploids in various species of Avena. X-irradiation at dose rates of 150 to 300 roentgens was found to be best for monosomic production in A. sativa var. Garry and Rodney. X-irradiation of A. barbata and A. strigosa panicles induced a number of aneuploids, particularly in A. barbata, where frequency increased with dose. Monosomic plants with 27 chromosomes were sterile, but 4 plants with 27 chromosomes plus a telocentric, set seed. Deficiencies in A. strigosa were either lethal or sterile.

Schulenburg (72) treated the seeds of oats, variety Borreck, with X-rays (3,000 roentgens) and ethyl methanesulphonate (EMS) (10 hours with 3.0% solution) to induce aneuploids. The percentages of aneuploids recorded following X-rays and EMS treatments were 13.6 per cent and 4.4 per cent, respectively. He investigated 10 monosomic lines.

Monosomics were only slightly reduced in plant height in comparison to disomics; however, their fertility was markedly reduced. Nullisomics in most cases were not studied, but where studied, they were weak and sterile. The percentage of nullisomics in different monosomic lines varied from 0 to 55.0 per cent. Two of the monosomic lines were cytologically examined in somatic metaphases. The missing chromosome in line B, which was not a fatuoid but showed a partial asynapsis with 7 II + 26 I, was identified as a subterminal chromosome falling in group 16 to 20. The frequencies of nullisomics and monosomics in monosome B were 20.0 per cent and 80.0 per cent, respectively. A submedian chromosome was found to be missing from monosome G which gave the highest frequency of nullisomics (55.0%). No correlation was found between univalent transmission rate and frequency of nullisomic plants in progenies of different monosomic lines

Dyck and Rajhathy (14), in a breeding program to incorporate crown rust (P. coronata sp. avenae) resistance from A. strigosa into cultivated varieties of A. sativa, obtained two lines with 44-chromosomes, which consisted of a hexaploid chromosome complement plus a pair of chromosomes from the As genome of A. strigosa. This extra chromosome was identified as M-4, according to the Rajhathy (64) karyotype, and carried the gene Pc-15 for resistance to races 264 and 294 of crown rust. They pointed out that the pairing of the M-4 chromosome of A. strigosa with that of A. sativa was low, indicating evolutionary differentiation.

Chang and Sadanaga (6) induced six monosomic lines (A, B, C, D, E, F) in A. sativa with X-rays. These monosomic lines were crossed to hexaploid oats varieties, species, and mutants, to determine if certain

genes were associated with the critical chromosomes. By using the above method the crown rust gene L of Landhafer and a gene for seed luminescence were located on monosome D, a gene for "netting leaf" designated as nt-1 located on A, a gene for "necrotic leaf" designated as nl located on B, and a gene for alboviridis designated as av on E. From their data we conclude that monosome F was the same as monosome-c reported earlier by Nishiyama (51, 52).

Continuing the above project, Chang and Sadanaga (7) studied the breeding behavior and morphology of the six monosomic lines. In progenies of selfed monosomes, the frequencies of disomics ranged from 3.1 to 15.4 per cent, monosomics from 84.3 to 95.4 per cent, and nullisomics from 0 to 6.0 per cent. Great variation in univalent transmission rate was observed from both the male and female gametes. The univalents present in above six monosomes did not correspond to any of the three pairs of satellited chromosomes or one pair of shortest chromosomes. The workers concluded that the C, D, and F monosomes were definitely different from the other monosomes and also from each other. None of the monosomes were identified karyotypically.

Lafever and Patterson (35) reported that nullisomic condition caused male sterility in a sib line of variety Clintland 60 of A. sativa. Univalent transmission in F_1 plants from crosses between male sterile nullisomics and normal disomics was found to be similar in ovule and pollen and averaged 6.03 per cent. F_2 populations from above F_1 plants yielded nullisomics, monosomics, and disomics in the ratio of 4.85:0.95:0.05, respectively. Univalent chromosome had a submedian centromere. The seed set on individual nullisomic plants varied from 0 to 55.0 per cent, and cooler temperatures promoted higher seed set.

In a recent investigation, Nishiyama and Tabata (56) searched for specific factors controlling chromosome pairing at tetraploid level ($AA\acute{A}\acute{B}$) and speculated that the functions of synaptic factors on the c-chromosome of the hexaploid species might be substituted by a synaptic factor of the tetraploid species. To explain this assumption, they crossed A. barbata (2n=28, $AA\acute{A}\acute{B}$) with a fatuoid heterozygote (2n-1=41, AABBCC-c) of A. sativa, which was the monosomic for the c-chromosome. The two types of F_1 plants, i.e., 35-chromosome plants ($AAB\acute{A}\acute{B}C$) and 34-chromosome plants ($AAB\acute{A}\acute{B}C$-c) obtained, were compared in their morphological characteristics and chromosome pairing. The 34-chromosome plants were shorter in plant height and showed the wild grain characters, because of the absence of c-chromosome, whereas 35-chromosome plants were normal. With regard to chromosome pairing, no differences were found between the two types in the average number of pairs or of chiasmata per cell at metaphase I. This convinced them that meiotic pairing between homologous chromosomes from the two species took place in the absence of synaptic factors of A. sativa and that chromosomes paired in the presence of the synaptic factors of A. barbata. They concluded from the above results that the present hexaploids have lost physically or functionally the synaptic factors in the A and B genomes and are dependent for their normal pairing upon the synaptic factors in the C genome.

Gauthier and McGinnis (16) associated "kinky neck" with chromosome 20 of A. sativa in a spontaneous monosomic of variety Garry. Plants monotelocentric for short arm also exhibited the extreme kinkiness of the nullisomics, suggesting that the long arm carried the gene for normal neck. Nullisomics were sterile and monosomics reached

35.0 per cent seed set under field conditions. The selfed monosomic-20 produced 5.0 per cent disomics, 84.9 per cent monosomics, and 10.1 per cent nullisomics. This distribution was explained by a low transmission rate of the univalent (9.0%), a marked certation effect, and a high lethality of nullisomic zygotes. Monotelocentrics occurred at a frequency of 2.5 per cent; their meiotic behavior was similar to that of univalent.

Hacker and Riley (21), based on phenotype and in some cases karyotype studies, grouped their Sun II monosomics and the derived nullisomics of A. sativa in 13 distinct classes (I-XIII). By interline crossing, four lines I, II, III, and XI were shown to be distinct. They suggested that in addition, by karyotype analysis three other lines, VIII, XII, and XIII, could be recognized as distinct from each other and from those distinguished by crossing. Thus they felt that there were seven unequivocally distinct monosomic lines in the Sun II material studied. Some nullisomics were associated with certain morphological characters, which have been associated with nullisomics involving different chromosomes in varieties Garry, Russell, and Rodney of A. sativa by other workers. Three distinct classes of nullisomics exhibited a high degree of pairing failure. Asynaptic behavior of the fatuoid nullisomic IV was the same as monosome-c reported by Nishiyama (51, 52). Nullisomic VIII formed o to 9 II and 40 to 22 I, and bivalents were mostly rings. Nullisomic XII was asynaptic and formed 40 I. The chromosomes involved in VIII and XII were 2 and 1, respectively, both satellited of the A genome according to the Rajhathy (64) karyotype. Selfed monosomics for these two lines segregated disomics, monosomics, and nullisomics in the ratios of 0.03:0.94:0.03 and 0:0.94:0.06, respectively.

Monosomics are not confined by any means to oats and have been reported in other organisms as well. Thus Clausen and Goodspeed (10, 11) reported two monosomics in Nicotiana tabacum for the first time, and one of them, haplo-C (then called corrugated), involved the chromosome in which the basic color factor wh was located. Olmo (57) reported that the transmission rate of monosomic condition through female and male gametes in Nicotiana tabacum varied a great deal for different monosomes. Later Clausen (8), and Clausen and Cameron (9) isolated all 24 monosomic lines in Nicotiana tabacum. Most of these monosomic lines were obtained by crossing asynaptic females by normal males. The writers pointed out that all monosomics differed from normal and from one another in a specific ensemble of quantitative morphological factors. Because of the marked differences in size and shape of the chromosomes it was easy to identify them. Ovular abortion rates of certain monosomics reached a high level of about 80.0 per cent as with pollen samples. Mallah (41) used monosomics to identify chromosomes involved in translocations in Nicotiana tabacum.

Sears (73, 76, 77) laid the foundation stone for systematic monosomic analysis in bread wheat, Triticum aestivum var. Chinese Spring, and from a series of investigations isolated all the 21 monosomic lines along with their derivatives. Most of the monosomics were obtained by pollination of haploids with diploids and from the partially asynaptic nullisomic III. In the offsprings of monosomic plants many telocentrics and isochromosomes were observed occurring mainly following misdivision of the univalents. Most of the nullisomics were reported to be stable except nullisomic III, which was

partially asynaptic. In general, nullisomics were mostly of reduced vigor and fertility but all survived to maturity and were at least partially fertile either as males or females, or both. Most of the nullisomics could be distinguished from normals by distinctive plant and spike characters. Frequencies of nullisomic plants ranged from 7.6 to 0.9 per cent. He suggested that certain genes in wheat had originated through mutations at loci that were not essential, because they were duplicated on other chromosomes.

Interpreting the results of nullisomic-tetrasomic compensations, Sears (77) divided the 21 pairs of chromosomes into seven groups, each of three pairs. Chromosomes in the same group had marked similarities in their genetic behaviors, whereas those in other groups did not have these similarities. When 21 pairs were classified on the basis of the parental diploid species from which they were derived, every group had one pair in every genome and every genome one pair in each group. The conclusion drawn indicated that pairs in the same group represent homoeologous chromosomes of the different genomes.

Monosomic lines and their derivatives produced and established by Sears (77) in Chinese Spring have been used to locate genes in hexaploid wheat by various workers (22, 28, 36, 37, 38, 78, 80). Morrison (48) described the behavior of univalents in wheat monosomics. In all monosomics 20 II + 1 I were observed in 97.0 per cent of the pollen mother cells (PMCs). The univalents either divided or misdivided and either lagged or did not lag at either first or second telophase. Isochromosomes arose directly through misdivision at meiosis and not indirectly through non-disjunction in pollen grains. Behavior of isochromosomes and univalents were also discussed by Sears (74, 75).

Kimber and Riley (31) speculated that aneuploids in a population may result in the fixing of the misdivision products, the transmission of the non-recombinant chromosome, and the limitation of recombination in chromosomes which become monosomic.

MATERIALS AND METHODS

Resistant mutant lines from Avena byzantina C. Koch var. Victorgrain provided the material from which monosomic lines were extracted. The material was provided by Dr. A. T. Wallace, Geneticist and Head, Plant Science Section, University of Florida. By using Co^{60} -rays, thermal neutrons and certain chemicals, Wallace (p.c.) induced approximately 600 morphologically distinct lines, showing varying degrees of resistance to Helminthosporium victoriae M. and M. The basis for monosomic induction in irradiated or chemically treated materials is as follows: induced dicentric chromosomes become involved in the breakage-fusion cycle and are usually lost during the early cell divisions of the embryo. If such cells happen to give rise to the floral structures, monosomic seed may be produced. Eighty-four resistant mutant lines from the above material were examined cytologically and the monosomics for this study isolated.

Screening of Monosomic Lines

Plants grown in the Spring of 1963 at Aberdeen, Idaho, were used for the initial screening purposes. Young panicles were fixed in Carnoy solution (6:3:1) for 48 hours, transferred to 70 per cent ethanol, and kept under refrigeration until used. Pollen mother cells (PMCs) were squashed in a drop of aceto-carmine stain and slides prepared. For greater efficiency, first of all the presence or

absence of micronuclei at the telophase II (tetrad stage) was determined under 20 X objective and 10 X eyepiece. If micronuclei were observed, the presence of a univalent was confirmed by examinations of metaphase I and anaphase I at higher magnifications. After confirmation that a particular plant in a mutant line was monosomic, progenies were grown from such plants in different lines and their further stability was checked. Seeds from only selfed monosomic plants of different monosomic lines were used for further studies.

Breeding Behavior

One hundred seeds from one selfed monosomic plant of each monosomic line were planted in 6 inch pots with 2 seeds per pot. The growing media consisted of 2 parts soil, 1 part peat, and 1 part perlite. A 10-10-10 fertilizer was added at the rate of 2,000 lbs./acre. Liquid fertilizer 20-20-20 was added at the rate of 1 tablespoonful to the gallon of water, 3 times during the entire growing period. The plants were grown in growth rooms, the settings of which are presented in Table 1.

Table 1. Growth room settings

Growing period	Night temp. (°F)	Day temp. (°F)	Day length (hours)	Relative humidity (%)
Apr. 21 to May 16, 1965	70	55	10	50
May 16 to May 31, 1965	75	65	14	50
May 31 to July 26, 1965	75	65	16	50

Young panicles of the proper size were collected from each plant in each line and fixed in Carnoy solution. The chromosome number of each plant was determined by examination of PMCs, at diakinesis and metaphase I stages. This information furnished the frequencies of disomics (2n=42), monosomics (21-1=41), nullisomics (2n-2=40), and any other types. Germination percentage in each line was determined by counting the number of young seedlings three weeks after the planting date. The fertility of different monosomics and their derivatives was determined by counting the seed set in the primary florets. To determine the data on mature plant height, the longest tiller of the desirable number of plants in each line was measured in centimeters.

Karyotype Analysis

For karyotype studies, dehulled seeds of selfed monosomic plants from each line were soaked for two hours in distilled water and then placed in petri dishes on a layer of 3 mm glass beads with 7 ml of distilled water. The petri dishes were kept in a germinator at 72^{o}F. One of the procedures outlined by Melnyk and Unrau (47) was used with some modifications for slide preparations. The root tips were collected 42 hours after soaking and pretreated in monobromonaphthalene at room temperature for four hours before fixing in Carnoy solution. Roots were left in the fixative at 24 hours. Then the root tips were hydrolyzed in 1 N. HCl at 62^{o}C for 13 minutes, washed with distilled water, and transferred to Feulgen reagent. After the proper staining was attained, the root tips were squashed in aceto-carmine. Temporary slides were prepared by using sealing wax. These slides can be stored at least for three days without deterioration. Chromosome

counts were made under 40 X high dry objective using a 10 X eyepiece in at least five cells. Photomicrographs of the best cells were taken. Two measurements of each chromosome were taken from the photographs.

Chromosomes cut out from the photograph were paired based on their total length, arm ratio, and morphology. Paired chromosomes, according to the presence of satellite and position of centromere were assigned to satellited (SAT), median (M), submedian (SM), and subterminal (ST) groups. A pair of dividers and a millimeter scale were used for taking the measurements of the chromosomes. A representative idiogram was prepared from the best karyotype. The system proposed by Rajhathy (64) for karyotype arrangement was used.

Cytological Behavior of Selfed Monosomic Derivatives in Different Monosomic Lines

Panicles were fixed and slides prepared as described earlier. When a disomic, monosomic, nullisomic, or any other type was observed, detailed observations were made of the various stages of meiosis. Specifically, behavior of univalents in monosomics and pairing relationships in nullisomics received great attention. To determine the expected frequencies of disomics, monosomics and nullisomics, the transmission rate of the univalent was calculated by using the method proposed by Nishiyama (51). This was done by counting the number of tetrads having 0, 1, 2, 3 or more micronuclei. If a tetrad had no micronuclei, two of its microspores were supposed to have 21 chromosomes and two 20 chromosomes. Similarly, the presence of one micronucleus indicated one microspore with 21 chromosomes and three with 20 chromosomes, and two or more micronuclei in one tetrad indicated all microspores had 20 chromosomes. By using these univalent transmission

rates from the male side, and assuming a similar transmission from female side, the frequencies of disomics, monosomics, and nullisomics were calculated for different monosomes.

Morphology and Association of Characteristics

Morphology of nullisomic plants in each monosomic line is described. By morphological and cytological observations of various types of plants in selfed monosomic progenies, certain characters were associated with the particular chromosome involved.

Investigations Concerning Vb Locus

All the monosomic lines isolated here in A. byzantina and five others (A,B,C,D,E) obtained from Dr. Sadanaga, Ames, Iowa, along with Victorgrain, a susceptible variety to H. victoriae, were grown under field as well as in air-conditioned greenhouse conditions during the year 1963-64. Monosomes obtained from Iowa were from a variety, Cherokee of A. sativa, and were resistant to H. victoriae.

From each monosomic line several monosomic plants were identified cytologically and were used as female parents in crosses with Victorgrain. Hybrid seeds obtained were stored for about three months to overcome their dormancy. Procedures of seed germination and chromosome counts from individual plants were the same as described for karyotype preparation. All plants were grown in the growth room as described earlier. After determination of chromosome number only monosomic seedlings were saved and grown to maturity. It was assumed that all monosomic plants so obtained were hybrid, but the possibility of a few selfed ones was not ruled out.

F_2 seeds obtained from the above hybrid monosomic plants were germinated and treated with toxin produced by H. victoriae; segregation ratios were then observed. The petri dish method being used in the Plant Science Laboratory was used for testing the reaction of F_2 seeds to toxin as described below.

Toxin itself is a secretion product from the fungus, H. victoriae and duplicates the symptoms produced by the fungus as first reported by Meehan and Murphy (46). Pringle and Braun (61) isolated the toxin from cultures of H. victoriae and after analysis reported it to be a penta-peptide complex. When it was subjected to a mild alkali treatment and hydrolysis, the products were asparatic acid, glutamic acid, glycine, valine and one of the leucines, and a new base victoxinine (C_{17} H_{29} NO).

Toxin used in the present investigation was obtained through the courtesy of Dr. H. H. Luke, Plant Pathologist, United States Department of Agriculture, Agricultural Experiment Station, University of Florida, who homogenized liquid cultures of H. victoriae and filtered the homogenate, presenting the filtrate as a toxin solution. To determine the activity of the toxin filtrate, a bioassay was conducted by growing susceptible Victorgrain seeds in serial dilutions of the filtrate, as described below, and measuring the root lengths at the various concentrations. The lowest concentration of the toxin which stopped all growth of Victorgrain roots was used in the segregation tests. That concentration was one per cent of the stock obtained from Dr. Luke.

Dehulled F_2 seeds were presoaked in distilled water for two hours and then planted in plastic refrigeration boxes between two wet blotters. These boxes were kept in the germinator at 72°F for 24 hours, at which time root lengths varied from 2 to 5 mm and the seeds were

ready to be placed in the toxin. Seeds having 2 to 5 mm root length were placed on a layer of 3 mm glass beads in 10 cm petri dishes, containing 7 ml of 1 per cent toxin solution. These plates were kept in a germinator at 72°F and the segregation ratios determined at the end of 48 hours. At this time resistant seedlings had long, white roots and susceptible seedlings had brown roots less than 10 mm long (Figures 1, 2).

The principle of monosomic analysis with regard to Vb locus is described below. Following is a diagramatic representation which reveals the manner of univalent transmission from a monosome to the monosomic F_1 hybrid, in a cross between monosomic and normal variety.

Parents	Monosome	X	Variety
	20 II + 1 I		21 II
Gametes	21 I or 20 I		21 I
F_1		20 II + I	

Assuming that the genotypes of resistant and normal susceptible variety Victorgrain are vbvb and VbVb, respectively, the results of a critical cross can be presented as follows:

Parents	Monosome	X	Victorgrain
	vb -		Vb Vb
Gametes	vb or -		Vb
F_1		Vb -	

F_2	Vb Vb	disomic (susceptible)
	Vb -	monosomic (susceptible)
	- -	nullisomic (resistant)

Since the frequencies of the nullisomic plants in a selfed monosomic progeny varies with different monosomes, a deviation from the normal 3:1 ratio would be expected if the critical chromosome is involved. In cases where the univalent transmission rate is 50 per cent, resulting in 25 per cent nullisomic plants, it would be impossible to distinguish this deviation from the normal 3:1 ratio, unless the chromosome number of the recessive class were to be cytologically checked. If the recessive class consists of all nullisomic plants, the dominant gene under test is considered to be located on the critical chromosome.

EXPERIMENTAL RESULTS

Screening of Monosomic Lines from Mutant Lines

From 84 resistant mutant lines cytologically examined for deficiency of a whole chromosome and identified by the presence of 20 pairs plus a univalent (20 II + 1 I) at diakinesis and metaphase I and by micronuclei at telophase II in pollen mother cells (PMCs), 8 monosomic lines were obtained. These lines are listed in Table 2 along with the treatments they received.

Table 2. Monosomic lines and their original mutagenic treatments

Monosomic lines	Moisture contents of the seeds treated (%)	Treatments
19-3-1*	10.0	40 Kr. Co^{60} γ
25-2-1	10.0	50 Kr. Co^{60} γ
136-2-4*	10.0	Thermal neutrons for 4 hours
213-3-1*	3.5	5 Kr. Co^{60} γ
213-5-4	" "	" "
213-8-9	" "	" "
221-7-8*	10.0	Soaked 2 hrs. in .07% ethyleneimine
473-6-6*	2.3	8 Kr. Co^{60} γ

*Only the starred monosomic lines were chosen for the detailed investigations.

The 8 monosomic lines among the 84 lines examined constitute a frequency of 9.52 per cent.

Breeding Behavior

Germination percentage

Seeds of selfed monosomic plants of the five monosomic lines were grown under growth room conditions as described earlier. To determine whether there was any significant difference in germination percentage in different lines, the number of young seedlings were counted three weeks after planting. The data are presented in Table 3.

Table 3. The germination percentage of seeds of selfed monosomic plants sown on April 21, 1965 in growth rooms

Monosomic lines	No. of seeds planted	No. of seeds germinated	% germination
19-3-1	100	96	96.00
136-2-1	100	90	90.00
213-3-1	100	96	96.00
221-7-8	100	99	99.00
473-6-6	100	84	84.00

The data in Table 3 clearly show that the germination percentages for all the monosomic lines were high with little difference between lines.

Observed frequencies of derivatives of selfed monosomic plants in different monosomic lines

Progenies of selfed monosomic plants presented in Table 3 in each of the monosomic lines were used for determining the frequencies of disomic, monosomic, nullisomic, and any other type of plants. Since weakness, retarded growth, sterility, and certain markers are usually associated with nullisomics, it is possible to identify them by observation

of morphological characteristics. Judged on the basis of such characteristics, the frequencies of nullisomics in different monosomic lines are presented in Table 4.

Table 4. Frequencies of nullisomics based on morphological characteristics

Monosomic lines	No. of plants	No. of nullisomics	% nullisomics
19-3-1	94	5	5.31
136-2-1	90	6	6.66
213-3-1	93	54	58.06
221-7-8	99	42	42.42
473-6-6	82	3	3.65

The actual frequencies of nullisomics (Table 9) as determined by cytological examination of each plant in each monosomic line agreed only with those of 213-3-1 and 221-7-8 in Table 4. These two lines had very distinct morphological markers associated with nullisomics, which helped to estimate the nullisomic frequencies which approached those determined by cytological examinations.

Although the estimation of nullisomic frequencies by morphological characters saves time and labor, its accuracy is not certain. Therefore their identity should always be checked by cytological examinations. Fruthermore, not only is the frequency of nullisomics important in cytogenetical investigations, but also the same can be said for the frequencies of monosomics and disomics. Since it is impossible to distinguish monosomics and disomics morphologically in this case, cytological examination was used to determine the exact

frequencies of disomics, monosomics, nullisomics, and any other types in the selfed monosomic progenies. The results are presented in Tables 5, 6, 7, and 8 along with fertility and mature stem height data for each line. The chromosome numbers were determined in the pollen mother cells (PMCs) in all cases. Since karyotype analysis, discussed in the next section, revealed that line 136-3-1 was the same as 19-3-1, the former is omitted from further discussions.

To compare the frequencies and fertilities of disomic, monosomic, and nullisomic plants in progenies of different selfed monosomics, the data from Tables 5, 6, 7, and 8 are summarized with omission of "other chromosomal types" in Table 9.

It is apparent from Table 9 that the monosomic lines varied considerably in the frequencies with which they segregated nullisomics and monosomics in their progenies; and this variation is attributed to variation in frequency of functioning 20 chromosome pollen grains. However, there seems to be some kind of selection for 20 chromosome eggs in monosomic lines 19-3-1 and 213-3-1. The complete absence of nullisomics and a very high frequency (85.10%) of monosomics in monosome 19-3-1, indicates inviability of deficient pollen grains but viability of deficient eggs. On the other hand, high frequencies of nullisomics in monosomics 213-3-1 (61.20%) and 221-7-8 (39.39%) indicates that both deficient pollen grains and eggs performed equally well.

Chang and Sadanaga (7) have suggested the use of frequencies of nullisomic plants in progenies of selfed monosomics as a method for distinguishing one monosome from others. Thus nullisomic frequencies of 0, 61.29, 39.39, and 15.85 per cent (Table 9) in monosomes 19-3-1,

Table 5. Frequencies, fertilities, and mature stem height of plants having various chromosomal constitutions from progenies of a selfed monosomic plant in monosome 19-3-1

Chromosome No.	Frequencies		Fertilities			Mature stem height (centimeters)		
	No. of plants	%	Total no. of florets examined	No. of seeds set	% Fertility	No. of plants	Range	Mean
21 II	2	2.10	184	184	100.00	2	76.2-82.6	79.38
20 II + 1 heteromorphic bivalent	3	3.20	280	246	87.85	3	69.85-82.6	74.93
20 II + 1 I	80	85.10	3,692	3,061	82.90	38	69.85-93.98	80.52
20 II + a telocentric for long arm	6	6.42	349	30	8.60	5	62.23-67.31	63.75
20 II + a telocentric for short arm	1	1.10	72	36	50.00	1	73.66	73.66
20 II + a telocentric for long & short arm	1	1.10	91	26	28.57	-	- -	- -
20 II + 2 isochromosomes + a telocentric for long arm	1	1.10	57	51	89.47	1	51.31	51.31
20 II	0	0	0	0	-	0	- -	- -

Table 6. Frequencies, fertilities, and mature stem height of plants having various chromosomal constitutions from progenies of a selfed monosomic plant in monosome 213-3-1

Chromosome No.	Frequencies		Fertilities			Mature stem height (centimeters)		
	No. of plants	%	Total no. of florets examined	No. of seeds set	% Fertility	No. of plants	Range	Mean
21 II	0	- -	0	0	- -	0	- - - - -	- -
20 II + 1 I	36	38.71	1,622	1,437	88.59	21	52.07-74.93	61.80
20 II	57	61.29	490	0	0	45	25.40-50.80	40.16

Table 7. Frequencies, fertilities, and mature stem height of plants having various chromosomal constitutions from progenies of a selfed monosomic plant in monosome 221-7-8

Chromosome No.	Frequencies		Fertilities			Mature stem height (centimeters)		
	No. of plants	%	Total no. of florets examined	No. of seeds set	% Fertility	No. of plants	Range	Mean
21 II	3	3.03	309	298	96.44	3	68.58-74.93	21.96
20 II + 1 I	48	48.49	2,772	2,516	90.76	28	52.07-67.31	59.31
20 II + a telocentric for short arm	3	3.03	----- *					
20 II + an isochromosome for short arm	1	1.03				4	48.26-55.88	52.07
0-10 II + 40-20 I + telocentric for long arm	5	5.05	316	14	4.24	5	50.80-62.23	55.37
0-10 II + 40-20 I	39	39.39	800	28	3.50	37	36.83-52.07	43.43

*Those plants were used in certain crosses, thus selfed seeds could not be presented.

Table 8. Frequencies, fertilities, and mature plant height of plants having various chromosomal constitutions from progenies of a selfed monosomic plant in monosome 473-6-6

Chromosome No.	Frequencies		Fertilities			Mature plant height (centimeters)		
	No. of plants	%	Total no. of florets examined	No. of seeds set	% Fertility	No. of plants	Range	Mean
21 II	2	2.44	376	348	92.55	2	66.04-69.85	66.95
20 II + 1 I	53	64.63	2,502	2,086	87.87	38	63.50-86.36	74.75
20 II + a telocentric for long arm	14	17.07	630	447	70.95	11	58.42-85.09	73.53
20 II	13	15.85	344	127	36.91	9	17.78-74.93	49.81

Table 9. Percentage frequencies and fertilities (in parentheses) of disomic, monosomic, and nullisomic plants from progenies of a selfed monosomic plant in different monosomic lines

Monosomic Lines	% Frequencies and Fertilities					
	Disomics		Monosomics		Nullisomics	
19-3-1	2.10	(100.0)	85.10	(82.91)	0	(- -)
213-3-1	0	(- -)	38.71	(88.59)	61.29	(0.0)
221-7-8	3.03	(96.44)	48.49*	(90.76)	39.39	(3.50)
473-6-6	2.44	(92.55)	64.63	(87.77)	15.85	(36.91)

*Does not include the frequency of 9.11% for monotelocentrics and monoisosomics.

213-3-1, 221-7-8, and 473-6-6, respectively, clearly show that these lines are different from each other. Not only the nullisomic frequencies, but also the monosomic frequencies can be used in this case to distinguish the monosomic lines because of their different values.

A monosomic similar to monosome 221-7-8, i.e., causing fatuoid phenotypes, has been isolated in A. sativa by a number of workers (5, 7, 18, 21, 23, 24, 25, 51, 52) with the detailed investigations being those of Nishiyama (51, 52). He designated his line as monosomic-c, characterized by heterozygous fatuoidy. In the selfed progeny of a plant monosomic for the c-chromosome he observed frequencies of 37.7, 58.6, and 3.7 per cent for nullisomics, monosomics, and disomics respectively, figures which agree well with the frequencies of 39.39, 57.50, and 3.03 per cent observed in the present investigation. The frequency of monotelosomics and monoisosomics together was 9.11 per cent which has been included in the monosomic class for above comparison. Although the previous workers have reported monosomic-c from A. sativa, this is the first detailed report of a monosomic line causing fatuoid phenotypes in A. byzantina. The three other monosomic lines reported here, to the best of the author's knowledge, have not been reported elsewhere.

Fertility as judged by seed set varied from 92.55 to 100 per cent for disomics, 82.81 to 90.76 per cent for monosomes, and 0 to 36.91 per cent for nullisomics in different monosomic lines. Complete sterility and partial fertility (3.5%) of nullisomics in monosomes 213-3-1 and 221-7-8, respectively, were the direct result of meiotic pairing failure, which is discussed later. Of the different monosomic lines, nullisomics in 473-6-6 had the highest fertility (36.91%).

Out of 94 plants in monosomic line 19-3-1, not a single nullisomic plant was detected, so fertility data could not be presented.

Chromosomal constitution other than nullisomic, monosomic, and disomic in some lines gave information about genes influencing fertility. Thus in monosome 19-3-1 plants with 20 II + 1 heteromorphic bivalent showed 87.85 per cent fertility; 20 II + a telocentric for short arm, 50.0 per cent; 20 II + a telocentric for long arm, 8.60 per cent; 20 II + a telocentric for long and short arm, 28.57 per cent; and 20 II + 2 isochromosomes for short arm + a telocentric for long arm, 89.47 per cent (Table 5). Fertility of monotelocentric plants for long arm in monosome 221-7-8 was found to be 4.24 per cent (Table 7), and 70.95 per cent in monosome 473-6-6 (Table 8). The implications of these findings will be discussed later.

During the investigation it was observed that plant height between different monosomic lines and also within lines showed considerable variation. To investigate the relationships between chromosome number and plant height, the longest tiller of the desirable number of plants was measured. The data are presented in Tables 5, 6, 7, and 8. It is evident from the data in these tables that there was no appreciable difference in average mean stem heights between disomic plants of different monosomic lines. Monosomic plants in monosomes 19-3-1, 213-3-1, 221-7-8, and 473-6-6 had mean stem heights of 80.52, 61.31, 59.31, and 74.73 centimeters, respectively. Mean stem heights of monosomic plants in monosomes 213-3-1 and 221-7-8 were clearly shorter than those of 19-3-1 and 473-6-6. Nullisomic plants in monosomes 213-3-1, 221-7-8, and 473-6-6 had mean stem heights of 40.16, 43.43, and 49.81 centimeters, respectively, which were much shorter than the

respective sister monosomic plants (Figures 3, 4, 5). Great reduction in plant height was also noticed in monosome 19-3-1 for plants with 20 II + a telocentric for long arm, and 20 II pairs + 2 isochromosomes for short arm + a telocentric for long arm (Table 5).

Since all the plants were grown under identical controlled conditions, most of the differences in the above mentioned characteristics are attributed to the absence of the whole or partial chromosome in question.

Karyotype Analysis

Though Rajhathy and Morrison (66) pointed out that the karyotype proposed for A. sativa could be used for other hexaploid species, Gill et al. (17) were the first to report a detailed karyotype analysis of A. byzantina. They arranged and numbered the chromosomes according to the increasing lengths of their short arms. They reported two pairs of satellited chromosomes with their future confirmation an open question. During the process of karyotype analysis for identification of the missing chromosomes in different monosomic lines, efforts were also made to settle the number of satellited pairs of chromosomes in A. byzantina. Detailed descriptions of methods used for karyotype analysis are presented in "Materials and Methods." Photomicrographs of somatic metaphases and karyotypes made out of them for monosomes 19-3-1, 213-3-1, and 473-6-6 are presented in Figures 6, 7, 8, 9, 10 and 11, respectively. A common idiogram for all the lines is presented in Figure 14. Measurements of chromosomes for satellite lengths, arm ratios, and total and relative lengths for monosomes 19-3-1, 213-3-1, and 473-6-6 are listed in Tables 10, 11, and 12, respectively.

Table 10. Groups, chromosome number, lengths* of long arms, short arms and satellites, arm ratio, total and relative lengths of individual chromosome of monosome 19-3-1

Groups	Chrom. no.	Long arm	Short arm	Sat.	Arm ratio[1]	Total length	Relative length[2]
SAT	1	9.1	3.2	1.6	0.35	13.90	100.00
	2	6.4	3.4	1.9	0.53	11.70	84.17
	8	7.0	3.4	1.5	0.49	11.90	85.61
M	3**	9.3	8.4		0.90	17.70	100.00
	4	7.2	6.7		0.93	13.90	78.53
	9	6.5	6.0		0.92	12.50	70.62
	10	6.4	6.0		0.94	12.40	70.05
SM	5	8.7	5.8		0.67	14.50	95.39
	6	7.5	5.5		0.73	13.00	85.53
	11	9.2	6.0		0.65	15.20	100.00
	12	8.9	5.9		0.66	14.80	97.36
	13	7.8	5.6		0.72	13.40	88.15
	14	7.1	4.9		0.69	12.00	78.95
	15	5.6	4.1		0.73	9.70	63.81
ST	7	9.9	4.3		0.43	14.20	100.00
	16	8.9	4.3		0.48	13.20	92.95
	17	8.4	3.9		0.46	12.30	86.62
	18	8.0	4.0		0.50	12.00	84.51
	19	7.9	3.9		0.49	11.80	83.10
	20	7.6	3.7		0.49	11.30	79.57
	21	5.2	2.6		0.50	7.80	54.93

*All measurements are in mm. after 2900X magnification.
**Homologous chromosome of M-3 is missing.
[1]Arm ratio is short arm over the long arm
[2]Relative length is the per cent of the length of the longest chromosome in each group.

Table 11. Groups, chromosome number, lengths* of long arms, short arms and satellites, arm ratio, total and relative lengths of individual chromosome of monosome 213-3-1

Groups	Chrom. no.	Long arm	Short arm	Sat.	Arm ratio[1]	Total length	Relative length[2]
SAT	1	11.0	4.5	1.8	0.41	17.30	100.00
	2	10.4	4.7	1.7	0.45	16.80	97.00
	8	10.5	4.4	2.4	0.42	17.30	100.00
M	3	10.8	9.8		0.91	20.60	100.00
	4	8.0	7.5		0.94	15.50	75.42
	9	8.2	7.1		0.87	15.30	74.45
	10	7.7	6.6		0.86	14.30	69.58
SM	5	10.2	7.0		0.69	17.20	92.97
	6	9.9	7.2		0.73	17.10	92.43
	11	10.7	7.8		0.73	18.50	100.00
	12	10.8	7.2		0.67	18.00	97.29
	13	8.9	6.9		0.78	15.80	85.40
	14	8.5	5.8		0.68	14.30	77.29
	15	7.4	5.0		0.68	12.40	67.02
ST	7**	12.4	5.0		0.40	17.40	100.00
	16	11.2	5.5		0.49	16.70	95.98
	17	11.0	5.0		0.47	16.00	91.95
	18	10.4	5.0		0.48	15.40	88.50
	19	10.0	5.0		0.50	15.00	86.20
	20	9.3	4.5		0.48	13.80	79.31
	21	6.5	3.2		0.49	9.70	55.75

*All measurements are in mm. after 3200X magnification.
**Homologous chromosome of ST-7 is missing.
[1]Arm ratio is short arm over the long arm.
[2]Relative length is the per cent of the length of the longest chromosome in each group.

Table 12. Groups, chromosome number, lengths* of long arms, short arms and satellites, arm ratio, total and relative lengths of individual chromosome of monosome 473-6-6

Groups	Chrom. no.	Long arm	Short arm	Sat.	Arm ratio[1]	Total length[2]	Relative length
SAT	1	12.1	5.0	2.3	0.41	19.40	100.00
	2	10.1	4.8	2.4	0.48	17.30	89.17
	8	12.0	5.0	2.4	0.41	19.40	100.00
M	3	12.5	11.3		0.90	23.80	100.00
	4	10.1	9.1		0.90	19.20	80.67
	9	10.0	9.0		0.90	19.00	79.83
	10	8.0	6.9		0.86	14.90	62.60
SM	5	12.5	8.1		0.65	20,60	89.57
	6	11.0	7.6		0.69	18.60	80.86
	11	14.0	9.0		0.64	23.00	100.00
	12	13.6	8.9		0.65	22.50	97.80
	13	8.9	6.8		0.76	15.70	68.26
	14	9.0	6.5		0.72	15.50	67.40
	15	8.9	5.7		0.64	14.60	63.48
ST	7	15.0	6.0		0.40	21.00	100.00
	16	14.0	6.5		0.46	20.50	97.62
	17**	13.0	- -		- -	- -	- -
	18	12.5	5.9		0.47	18.40	87.62
	19	12.0	5.8		0.48	17.80	84.76
	20	11.5	5.5		0.48	17.00	80.95
	21	8.0	4.0		0.50	12.00	57.14

*All measurements are in mm. after 3386X magnification.
**Homologous chromosome of ST-17 is missing.
[1]Arm ratio is short arm over the long arm.
[2]Relative length is the per cent of the length of the longest chromosome in each group.

Three pairs of satellited chromosomes were recognized as shown in Figure 12, a photomicrograph of monosome 213-3-1. Karyotype analysis of monosome 19-3-1 revealed the absence of the longest median chromosome, which is also the longest of all the chromosomes (Figure 7). This chromosome, designated as M-3, was assigned to the A genome. One arm of this chromosome is slightly longer than the other and in some cases seems to have a small constriction at the end. The arm ratio (length of short arm/length of long arm) for this chromosome was determined to be 0.903. This same chromosome was also found to be missing from monosome 136-2-4. In good preparations of somatic metaphase this chromosome can be identified with full confidence.

The missing chromosome in monosome 213-3-1 was found to be in the subterminal group and was designated as ST-7 in the A genome (Figure 9). The reasons for assigning it to the A genome are discussed in the next section. Even after continuous efforts, a good somatic metaphase suitable for karyotype analysis was not obtained for monosome 221-7-8. To give an idea of the chromosome's shape and size at somatic metaphase, a photomicrograph of the monosome 221-7-8 is shown in Figure 13. From meiotic studies it is clear (Figure 57) that the critical chromosome in monosome 221-7-8 belongs to the submedian group and may be either SM-12 or 13. In meiotic studies Nishiyama (51) found the critical chromosome to be submedian in monosome-c.

In monosome 473-6-6 the missing chromosome was subterminal. In the photomicrograph and karyotype only the long arm of the critical chromosome is present (Figures 10, 11). Since the subterminal chromosomes ST 16-20 are indistinguishable, any one of them may be the missing chromosome. Here ST-17 is chosen to be the missing one.

However, the subterminal chromosome in monosome 473-6-6 is definitely different from that of 213-3-1, the evidence for which is presented in the next section. A representative idiogram prepared from the karyotype of monosome 19-3-1 is presented in Figure 14, which can be used as standard idiogram.

That the monosomic lines are different from each other, as indicated earlier, is further supported by the results of karyotype analysis, except line 136-2-1, which had the same missing chromosome as monosome 19-3-1.

Cytological Behavior of Derivatives of Selfed Monosomics in Different Monosomic Lines

Determination of theoretical frequencies of disomics, monosomics, and nullisomics in progenies of selfed monosomic plants in different monosomic lines

For the above purpose the transmission rates of univalents in monosomic plants were determined for all the monosomic lines as described earlier. The data on univalent transmission rates are presented for different monosomic lines in Tables 13, 14, 15, and 16.

Univalent transmission rates from the male side for monosomes 19-3-1, 213-3-1, 221-7-8, and 473-6-6 were determined to be 19.07, 11.65, 9.32, and 21.37, respectively (Tables 13, 14, 15, 16). Assuming a similar univalent transmission rate from the female side, the calculation of expected frequencies of disomics, monosomics, and nullisomics for monosome 19-3-1, as an example, are shown in Table 17. Similar calculations for all monosomic lines are summarized, along with the observed frequencies of disomics, monosomics, nullisomics, and other chromosomal types in Table 18.

Table 13. Number of micronuclei in tetrads of monosomic plants of monosome 19-3-1 and calculation of univalent transmission rate

No. of micronuclei/tetrad	No. of tetrads	No. of gametes	No. of gametes with n=21	% gametes with n=21
0	82	328	164	50.00
1	119	476	119	25.00
2	149	596	0	0
3	20	80	0	0
4	1	4	0	0
Total	371	1,484	283	19.07

Table 14. Number of micronuclei in tetrads of monosomic plants of monosome 213-3-1 and calculation of univalent transmission rate

No. of micronuclei/tetrad	No. of tetrads	No. of gametes	No. of gametes with n=21	% gametes with n=21
0	93	372	186	50.00
1	117	468	117	25.00
2	336	1,344	0	0
3	84	336	0	0
4	20	80	0	0
Total	650	2,600	303	11.65

Table 15. Number of micronuclei in tetrads of monosomic plants of monosome 22l-7-8 and calculation of univalent transmission rate

No. of micronuclei/tetrad	No. of tetrads	No. of gametes	No. of gametes with n=21	% gametes with n=21
0	76	304	152	50.00
1	165	660	165	25.00
2	421	1,648	0	0
3	161	644	0	0
4	27	108	0	0
Total	850	3,400	317	9.32

Table 16. Number of micronuclei in tetrads of monosomic plants of monosome 473-6-6 and calculation of univalent transmission rate

No. of micronuclei/tetrad	No. of tetrads	No. of gametes	No. of gametes with n=21	% gametes with n=21
0	110	440	220	50.00
1	92	368	92	25.00
2	121	484	0	0
3	40	160	0	0
4	2	8	0	0
Total	365	1,460	312	21.37

The data presented in Table 18 clearly indicate that there is no agreement between expected and observed frequencies of disomics, monosomics, and nullisomics in any of the monosomic lines studied. Furthermore, it is practically impossible to determine the frequencies of plants with chromosomal types other than disomic, monosomic, and nullisomic by using univalent transmission rates.

Table 17. Expected frequencies of disomics, monosomics, and nullisomics from a selfed monosomic plant of monosome 19-3-1, with 19.07 per cent univalent transmission rate

Male gametes / Female gametes	n=21 (19.07%)	n=20 (80.93%)
n=21 (19.07%)	disome (21 II) 3.64%	monosome (20 II + 1 I) 15.43%
n=20 (80.93%)	monosome (20 II + 1 I) 15.43%	nullisome (20 II) 65.50%

Meiotic Behavior of Chromosomes in Derivatives of Selfed Monosomic Plants of Different Monosomic Lines

Monosome 19-3-1

In progenies of selfed monosomics, plants with the chromosomal constitutions listed in Table 5 were obtained. Disomic plants always formed 21 II, easily detectable at diakinesis and metaphase I (Figures 15, 16). All the meiotic stages proceeded normally and normal pollen grains were formed. Since disomic plants in all the monosomic lines showed similar chromosomal behavior, the latter are not discussed for

Table 18. Expected and observed frequencies of disomics, monosomics, nullisomics, and other chromosomal types, in different monosomic lines

Monosomic lines	% Disomics		% Monosomics		% Nullisomics		% Other types	
	E	O	E	O	E	O	E	O
19-3-1	3.64	2.13	30.87	85.11	65.50	0	-	12.90
213-3-1	1.36	0	20.59	38.71	78.05	61.29	-	0
221-7-8	0.87	3.03	16.91	48.49	82.22	39.39	-	9.11
473-6-6	4.57	2.44	33.61	64.63	61.83	15.85	-	17.07

E = Expected, O = Observed

the rest of the lines. Plants with 20 II + 1 heteromorphic bivalent (Figures 17, 18) also had normal meiosis, except in a few instances the telocentric from the heteromorphic pair formed a micronucleus. In no case did the univalent from the heteromorphic pair form a micronucleus. This might suggest that for the regular movements of the chromosomes at anaphase I, pairing of even one arm with its homologous chromosome is sufficient.

Monosomic plants normally formed 20 II + 1 I. The presence of a univalent was observed at diakinesis, metaphase I, and anaphase I. In most cases the univalent was off the metaphase plate and divided mitotically only after the other chromosomes moved towards the poles. These univalents either entered opposite daughter nuclei, or both entered the same nucleus. Generally at anaphase II they lagged behind and formed micronuclei and consequently were lost in the cytoplasm. Thus calculations showed that univalents were included in only 19.07 per cent of the male gametes. Figures 19, 20, 21, 22, 23, and 24 show the behavior of univalents at various meiotic stages. Figure 22 clearly shows the median position of the centromere. Abnormal behavior of univalents can be observed in Figures 23 and 24. It is evident from Figure 23 that the abnormal shape of univalent is associated with fragmentation of the nucleolus.

Plants monotelocentric for either short or long arms formed 20 II + a fragment, as is evident from Figures 25, 26, 27, 28, and 29. The telocentric arm behaved like a univalent in all respects except that few micronuclei were observed at late telophase II. This might indicate that the transmission rate of telocentrics into male gametes is higher than that for univalents. One of the plants had 20 II + 2

isochromosomes + a telocentric for long arm. The isochromosomes behaved like univalents except for being ring shaped at metaphase I (Figures 30, 31). Pairing of homologous chromosomes in all classes described here was normal. Because of the absence of the nullisomic plants their cytological behavior could not be examined. However, the absence of short and long arms in different plants did not show any effect on chromosome pairing (Figures 25, 27).

Monosome 213-3-1

Monosomic plants showed normal pairing with 20 II + 1 I (Figures 32, 33). The univalent in this line was found to be shorter than that present in monosome 19-3-1 (Figure 32). Figure 35 shows the subterminal position of the centromere at anaphase I. Behavior of univalents at different meiotic stages can be seen from Figures 34, 36, and 37. Univalent transmission rate to the male gametes was 11.65 per cent. Pollen grains formed were normal (Figure 38).

Nullisomic plants in this line were found to be very interesting from the point of view of pairing of homologous chromosomes. Chromosomes did not pair and remained single during the prophase stages, as can be seen from Figures 39, 40, and 41. During later meiotic stages, at metaphase I and anaphase I, they segregated to opposite poles in a very special manner. The 28 univalents moved to opposite poles in a group of 14 each, leaving 12 univalents in the middle of the pollen mother cell (PMC) (Figures 42, 43, 44, 45, 46, 47, 48). This unique and interesting behavior was confirmed by cytological examination of all 57 nullisomic plants. In some cases this grouping was not clear and univalent chromosomes were irregularly distributed all over the pollen mother cell (PMC) (Figure 49). Furthermore, it was noticed

that the 12 chromosomes in the middle of the cells behaved more like mitotic chromosomes than did the other 28 chromosomes. The second meiotic division, or homotypic division, continued very irregularly resulting in unequal distribution of chromatin materials to gametes (Figures 50, 51, 52). This resulted in the formation of sterile pollen grains (Figure 53).

It is postulated that the 12 chromosomes in the middle of the pollen mother cells (PMCs), which behave like mitotic chromosomes, are from the A genome of the hexaploid A. byzantina. Finally the missing chromosome in monosome 213-3-1 has a gene or genes controlling the normal pairing of all the chromosomes; its absence results in complete asynapsis.

Monosome 221-7-8

Monosomic plants regularly formed 20 II + 1 I, as observed at diakinesis and metaphase I. At metaphase I the univalent remained off the plate (Figures 54, 55), and further behavior of the univalent was similar to that described for the univalents of monosomes 19-3-1 and 213-3-1. At anaphase I the submedian position of the centromere was noticed (Figures 56, 57, 58). The univalent in this line appeared shorter than that of 19-3-1 and longer than that of 213-3-1. In some cells a constriction at the end of the long arm of the univalent was noticed, as can be seen from Figure 59. The univalents were included in only 9.32 per cent of the male gametes. Pollen grains from monosomic plants are shown in Figure 60.

Nullisomic plants in this line were as interesting as those of monosome 213-3-1. In the early meiotic prophase 20 loosely associated bivalents were observed (Figures 61, 62, 63, 64), but by late

diakinesis and metaphase I, most of the chromosomes had separated and formed univalents, leaving 0-10 II in various cells (Figures 65, 66, 67). Movement of chromosomes to opposite poles was found to be just opposite to that described for nullisomic 213-3-1. Thus 7 II of chromosomes moved faster at anaphase I, leaving 26 univalents in the middle of the pollen mother cells (PMCs) (Figures 68, 69, 70). It is proposed that the movements of the 7 II occurs because of the presence of a pairing gene in the A genome and none in the C and D genomes. The pairing gene in the C and D genomes is missing in the nullisome. These results were confirmed by examining pollen mother cells (PMCs) from all 39 nullisomic plants. Further divisions continued till tetrad formation with extreme irregularities (Figures 71, 72). Pollen grains formed were mostly sterile, but some normal and a few giant pollen grains, which stained with aceto-carmine as if they were fertile, were formed (Figures 73, 74).

Thus it is evident that this submedian chromosome also carries a gene or genes controlling a special phase of the pairing processes. Since homologous pairing was observed in the early prophase in this material, the use of the term desynapsis, instead of asynapsis, as used by various workers for the similar nullisomic line, is proposed to describe the pairing failure during later prophase and metaphase I stages.

The presence of 5 plants monotelocentric for the long arm and two plants monotelocentric for short arm further made it possible to locate the genes influencing pairing on one of the arms. The meiotic behavior of the five plants monotelocentric for the long arm was exactly like that of nullisomic plants, except that the former had 40

chromosomes plus a telocentric for long arm (Figures 75, 76, 77). However, plants monotelocentric for short arm showed perfect pairing, i.e., 20 II + a telocentric for short arm (Figure 78). The short arm behaved like a univalent in its division and movements (Figures 79, 80). This was further supported by complete pairing observed in pollen mother cells (PMCs) from a plant monoisosomic for short arms (Figures 81, 82, 83, 84). Nishiyama (51, 52) reported results opposite to these observations. The results presented here clearly indicate the presence of a pairing gene or genes on the short arm of the critical chromosome.

Monosome 473-6-6

Monosomic plants showed 20 II + 1 I at diakinesis and metaphase I and the behavior of the univalents can be seen from Figures 85, 86, 87, and 88. Anaphase I studies revealed the subterminal position of the centromere (Figure 88). The transmission rate of the univalents to male gametes was found to be 21.37 per cent, the highest percentage among all the monosomic lines.

Nullisomic plants formed 20 II as observed at diakinesis and metaphase I (Figures 89, 90, 91, 92, 93). Further meiotic stages proceeded very regularly, resulting in the formation of normal microspores (Figure 95). In rare cases in some cells, abnormally long chromosomes were observed at metaphase I (Figure 94).

Morphological Characteristics of Derivatives of Different Monosomes

It was not possible to distinguish monosomic plants from disomics in any of the monosomic lines except 221-7-8. In monosomic line 221-7-8, monosomic plants closely resembled the disomics in their morphological characteristics, but could be distinguished from the

latter by the presence of an awn on the first floret of the spikelets (Figures 100-2, 101-2). In monosomic line 19-3-1 plants with 20 II + 2 isochromosomes for short arm + a telocentric long arm, showed thick stems, equilateral inflorescence with short peduncles, and the lemma color was dark gray (Figures 96-4, 97-4). Nullisomic plants were clearly distinct from their monosomics or disomics. The morphological characteristics of nullisomic plants are described for the different lines and photographs of panicles and kernels are presented for nullisomics along with other types and Victorgrain (Figure 106) for comparison.

19-3-1

No nullisomic plant was obtained.

213-3-1

Nullisomic plants were very weak, approximately two-thirds as tall as monosomic plants, were profusely tillered, had narrow leaves, and in most cases panicles remained inside the sheaths. The panicles had short internodes and the florets had normal grain characteristics (Figures 98-2, 99-2). These plants remained green and were completely self-sterile.

221-7-8

Nullisomic plants were approximately one-half as tall as disomic plants, had strong stems, and stiff panicles. These plants were homozygous fatuoids, i.e., had a strong geniculate awn on every floret, and the sucker-mouth articulation surface surrounded by dense pubescence (Figures 100-4, 101-4). Crooked rachis base and yellowish leaf spots were also distinct markers (Figures 102, 103). These plants had 3.5 per cent seed set.

Plants monotelocentric and isomonosomic for the short arms had the same morphological characteristics as described for nullisomic plants (Figures 100-5, 101-5), whereas plants monotelocentric for the long arm had normal grain characters like monosomic plants (Figures 100-3, 101-3).

473-6-6

Nullisomic plants were weak, tiller numbers were reduced from 6-7 to 2-3, and were two weeks later in maturity than their monosomics or disomics. Floret bases were found to be dark green in color and a heavy awn development was noticed on lemma (Figures 104-3, 105-3).

Investigations Concerning Vb Locus

To investigate whether any of the four monosomic lines established here, and five monosomic lines (A,B,C,D,E) obtained from Dr. K. Sadanaga, Ames, Iowa, carry the vb locus on the critical chromosome, appropriate crosses were made and F_2 seeds were tested with H. victoriae toxin, according to the procedures described in "Materials and Methods." Since seed set did not occur in all the crosses attempted, the F_2 segregation ratios to the toxin are presented in Table 19 for only those monosomic lines where hybrid seeds were obtained.

If the vb locus is present on the critical chromosome in any of the monosomic lines, and plants nullisomic for the chromosome carrying the vb locus are resistant to toxin, the frequency of resistant plants in the F_2 segregation should be equal to nullisomic frequency in those lines. The data are presented in Table 20 for the above comparison.

It can be seen from Table 20 that there is no agreement between the observed and expected number of resistant plants. Thus none of

Table 19. F_2 segregation ratios of crosses between monosomic lines and Victorgrain (Vg) to H. victoriae toxin at 10^{-2} concentration

Parents used in crosses	No. of seeds placed	No. of susceptible seeds	No. of resistant seeds	Ratio Susc. : Res.
Vg x 19-3-1	180	135	45	3.00:1
Vg x 136-2-4	104	77	27	2.85:1
Vg x 213-3-1	105	78	27	2.89:1
Vg x A	35	28	7	4.00:1
Vg x B	29	25	4	6.25:1
Vg x C	45	38	7	5.42:1
Vg x E	152	112	40	2.80:1

Table 20. Observed percentages of resistant plants from F_2 segregation (Table 19), and expected percentages of resistant plants based on nullisomic frequencies in different monosomic lines

Monosomic lines	% Observed resistant plants	% Expected resistant plants (% nullisomics)
19-3-1	24.32	0
136-2-1	25.90	0
213-3-1	25.71	61.29
A	20.00	0
B	13.79	0
C	15.55	6.20
E	26.31	1.20

the monosomic lines tested involve the chromosome carrying the vb locus. This statement is further supported by the fact that three of the monosomic lines, namely 19-3-1 (136-2-1), 213-3-1, and E, show simple monogenic Mendelian segregation, indicating that both the homologous chromosomes are present. Deviations from a 3:1 ratio for lines A, B, and C of Ames may be the result of the small number of seeds or of chromosomal aberrations.

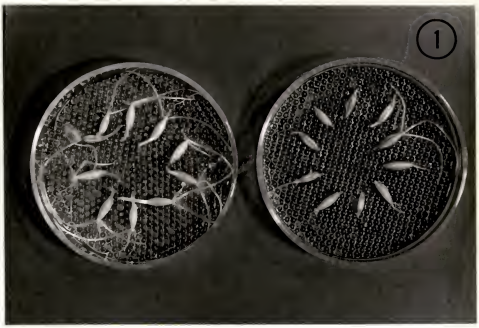


Figure 1. Segregation for resistance and susceptibility to Helminthosporium victoriae M. and M. toxin, in F_2 seedlings of a cross between monosome (resistant) and the Victorgrain (susceptible) variety of Avena byzantina C. Koch.

Figure 2. Close-up of resistant and susceptible seedlings from Figure 1.

Figure 3. Comparative view of the monosomic and nullisomic plants of monosomic line 213-3-1.

Figure 4. Comparative view of the monosomic and nullisomic plants of monosomic line 221-7-8.

Figure 5. Comparative view of the monosomic and nullisomic plants of monosomic line 473-6-6.


3
MONO
NULL


4
MONO.
221-7-8
NULLI.
221-7-8


5
NULLI.
473-6-6
MONO.
473-6-6

Figure 6. Photomicrograph of somatic metaphase of a monosomic plant of monosomic line 19-3-1.

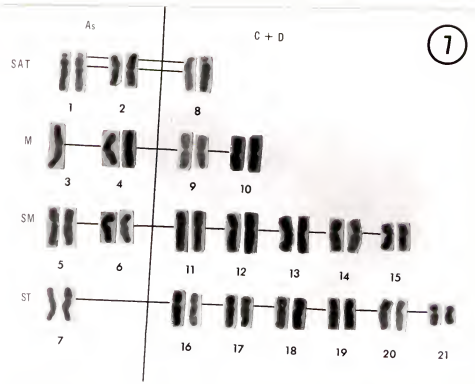


Figure 7. The karyotype of a monosomic plant of monosomic line 19-3-1 from Figure 6.

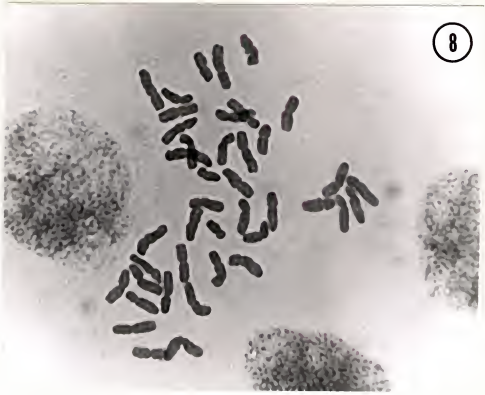


Figure 8. Photomicrograph of somatic metaphase of a monosomic plant of the monosomic line 213-3-1.

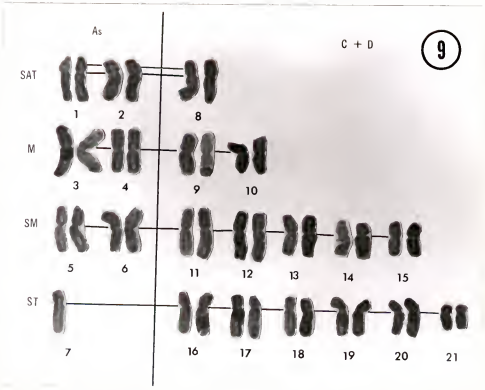


Figure 9. The karyotype of a monosomic plant of the monosomic line 213-3-1 from Figure 8.

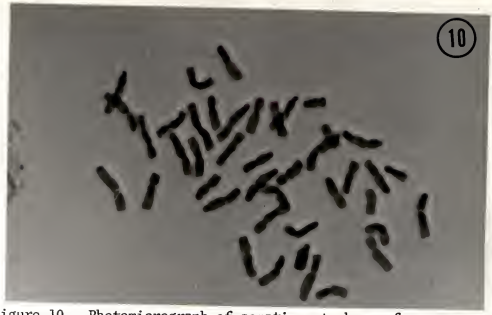


Figure 10. Photomicrograph of somatic metaphase of a monosomic plant in the monosomic line 473-6-6.

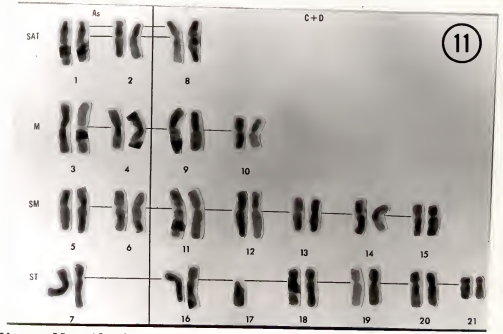


Figure 11. The karyotype of a monosomic plant of monosomic line 473-6-6 from Figure 10.

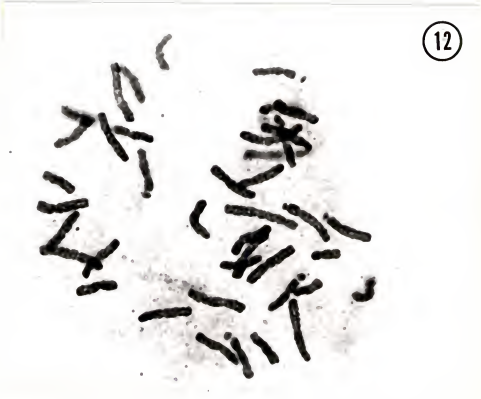


Figure 12. Photomicrograph of somatic metaphase of a plant from the monosomic line 213-3-1 showing three pairs of satellited chromosomes.

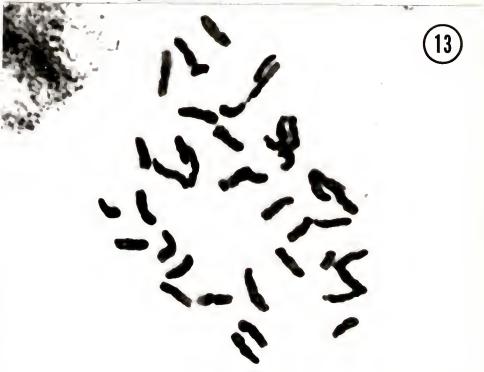


Figure 13. Photomicrograph of somatic metaphase of a monosomic plant in the monosomic line 221-7-8.

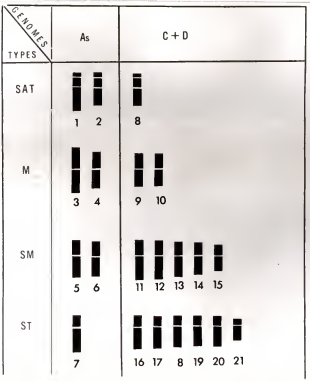
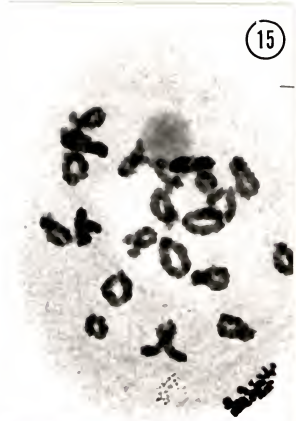


Figure 14. A representative idiogram prepared from the karyotype presented in Figure 7.

Figure 15. Diakinesis showing 21 II in a pollen mother cell of a disomic plant in the monosomic line 19-3-1.

Figure 16. Metaphase I showing 21 II in a pollen mother cell of a disomic plant in the monosomic line 19-3-1.

Figures 17, 18. Metaphase I showing 20 II + 1 heteromorphic bivalent in pollen mother cells of a plant in the monosomic line 19-3-1.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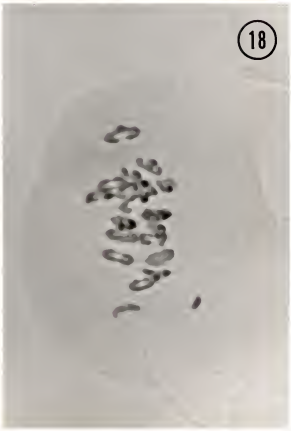
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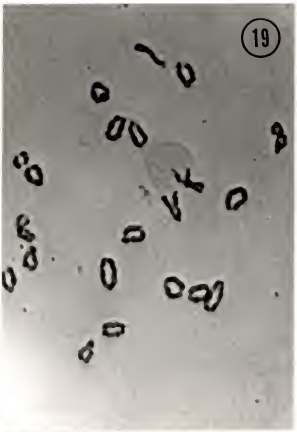
Figures 19, 20, 21, 22. Photographs showing behavior of univalents in pollen mother cells of a monosomic plant in the monosomic line 19-3-1. 19. Diakinesis 20 II + 1 I. 20, 21. Metaphase I, 20 II + 1 I. 22. Telophase I, median position of the centromeres in univalents.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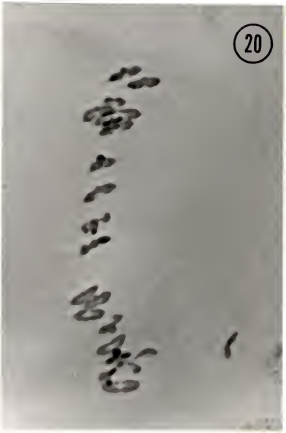
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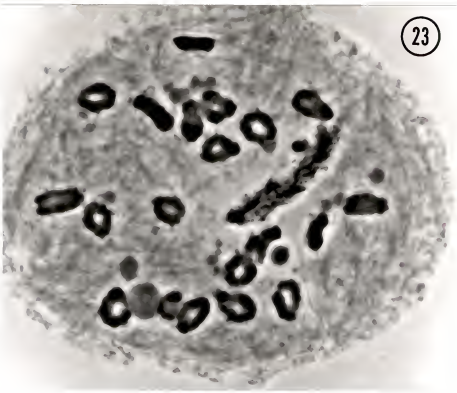
Figures 23, 24. Photographs showing abnormal behavior of univalents. 23. Diakinesis, 20 II + 1 I. Notice the abnormal shape of the univalent and the fragmentation of the nucleolus. 24. Telophase I with the univalent abnormally long and undivided.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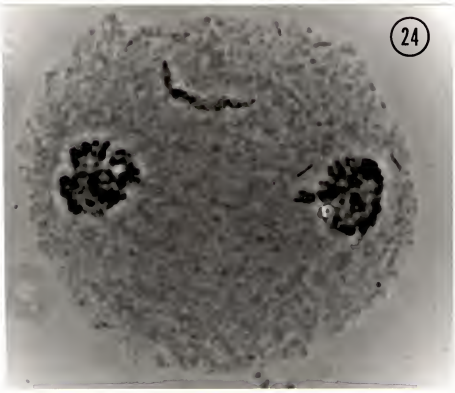
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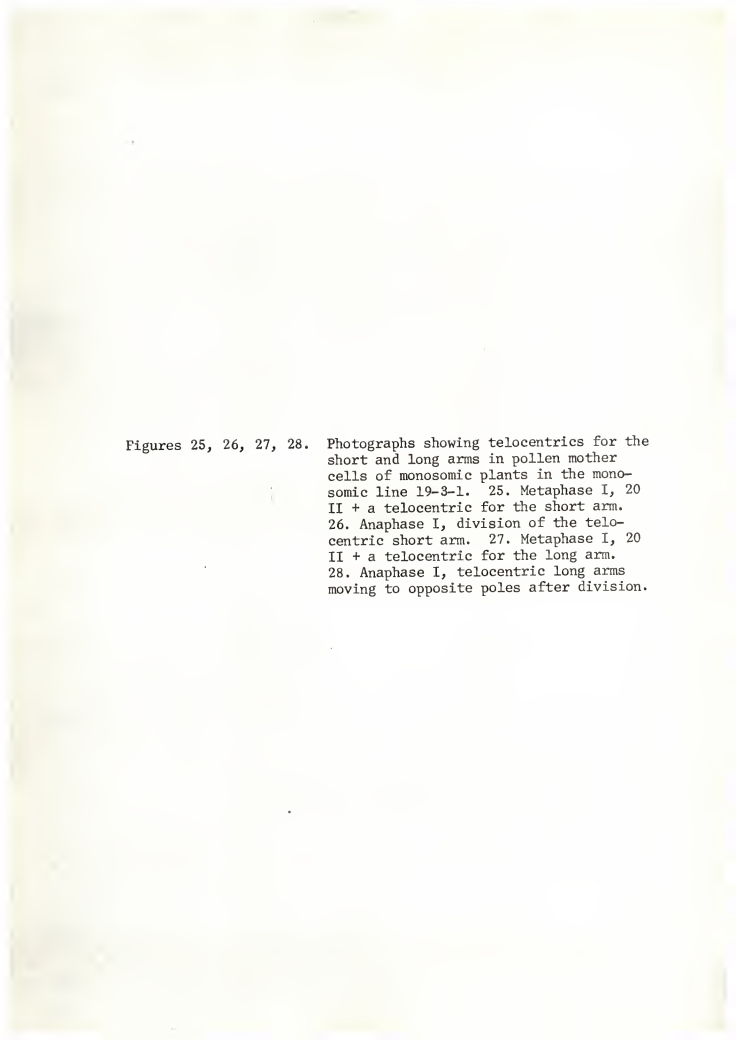
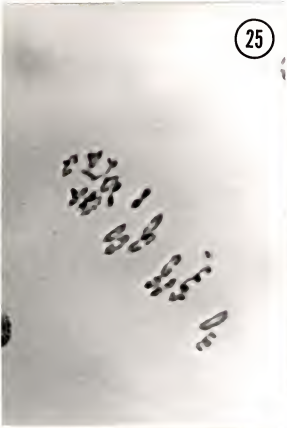


Figures 25, 26, 27, 28. Photographs showing telocentrics for the short and long arms in pollen mother cells of monosomic plants in the monosomic line 19-3-1. 25. Metaphase I, 20 II + a telocentric for the short arm. 26. Anaphase I, division of the telocentric short arm. 27. Metaphase I, 20 II + a telocentric for the long arm. 28. Anaphase I, telocentric long arms moving to opposite poles after division.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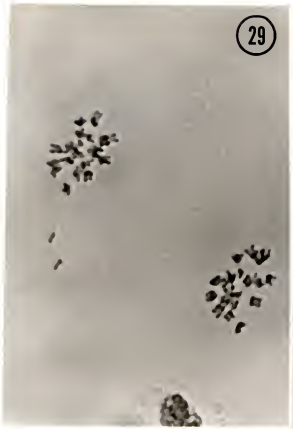
Figures 29, 30, 31. Photographs showing isochromosomes and telocentric in pollen mother cells of a plant in the monosomic line 19-3-1. 29. Anaphase I showing telocentric long arms moving to the same pole in a pollen mother cell of monosomic line 19-3-1. 30. Metaphase I, 20 II + 2 isochromosomes for the short arms + a telocentric for the long arm. 31. Late anaphase I, movements of divided isochromosomes and telocentrics.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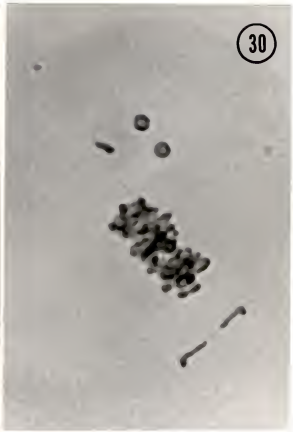
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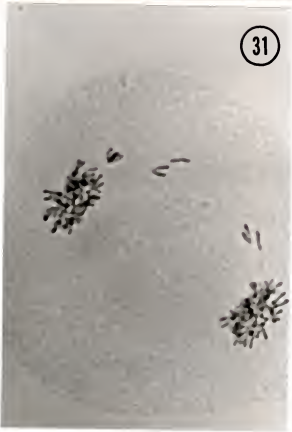
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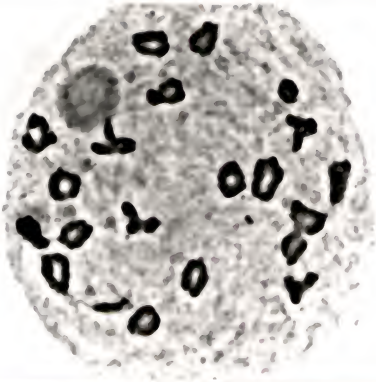
Figures 32, 33, 34. Behavior of univalents in pollen mother cells of monosomic plants in the monosomic line 213-3-1. 32. Diakinesis, 20 II + 1 I. 33. Metaphase I, 20 II + 1 I. 34. Anaphase I, division of univalent.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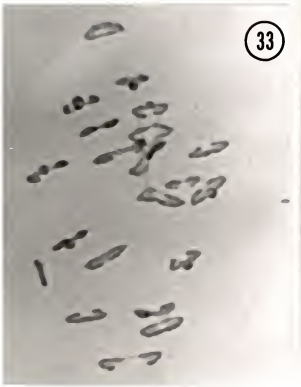
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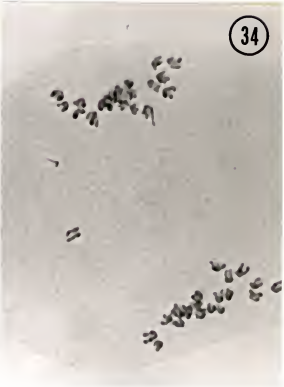
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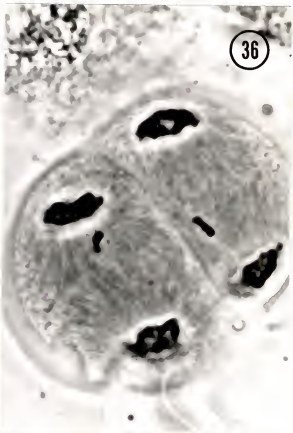
Figures 35, 36, 37, 38. Behavior of univalents in pollen mother cells of monosomic plants in the monosomic line 213-3-1. 35. Telophase I, movement of the divided univalent to opposite poles. Notice the subterminal position of the centromere in the univalent. 36. Telophase II, notice the lagging univalents. 37. Late telophase II, two micronuclei formations. 38. Normal pollen grains of a monosomic plant in the monosomic line 213-3-1.


35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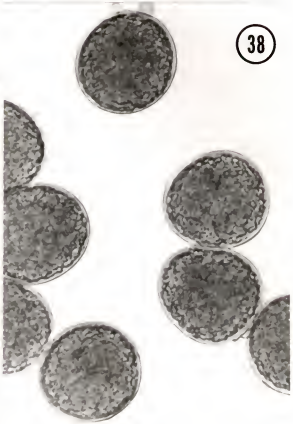
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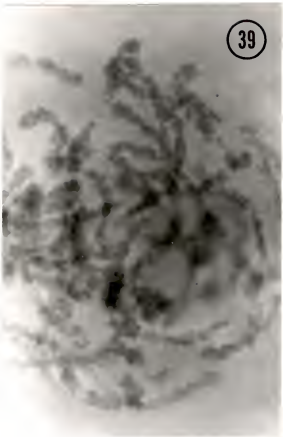
Figures 39, 40, 41. Early prophase I stages showing the pairing failure of homologous chromosomes in pollen mother cells of nullisomic plants in the monosomic line 213-3-1.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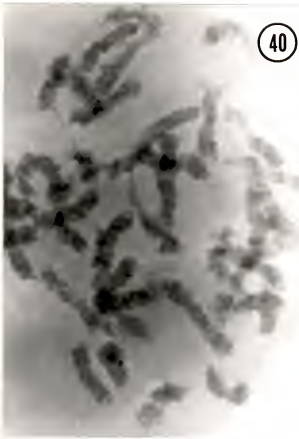
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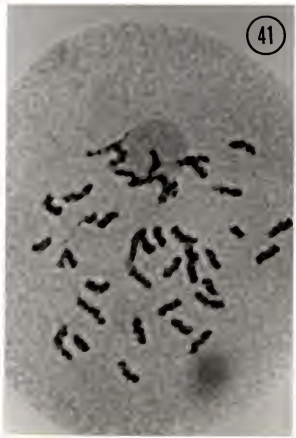
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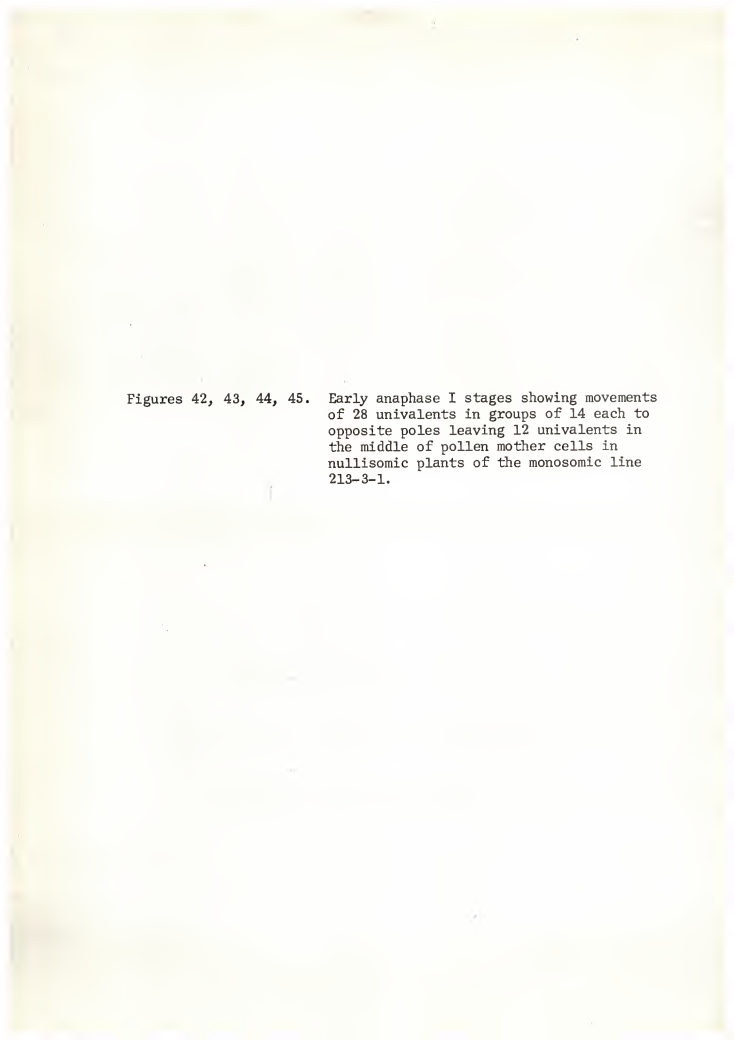
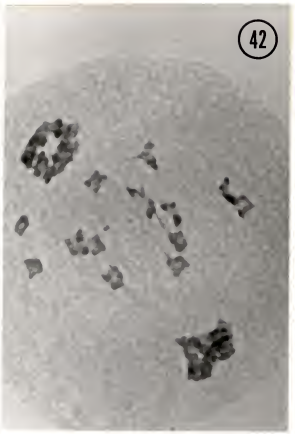


Figures 42, 43, 44, 45. Early anaphase I stages showing movements of 28 univalents in groups of 14 each to opposite poles leaving 12 univalents in the middle of pollen mother cells in nullisomic plants of the monosomic line 213-3-1.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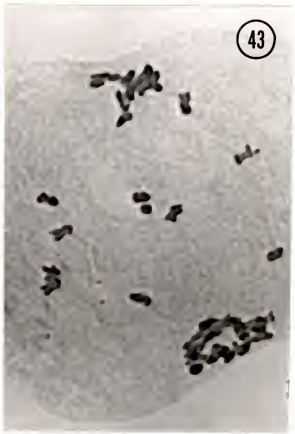
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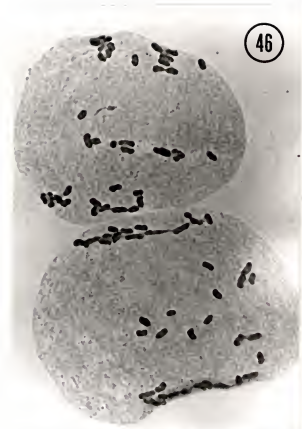
Figures 46, 47, 48. Early anaphase I stages showing movements of 28 univalents in groups of 14 each to opposite poles leaving 12 univalents in the middle of pollen mother cells in nullisomic plants of the monosomic line 213-3-1.

Figure 49. Early anaphase I showing irregular movements of the univalents in pollen mother cell of a nullisomic plant in monosomic line 213-3-1.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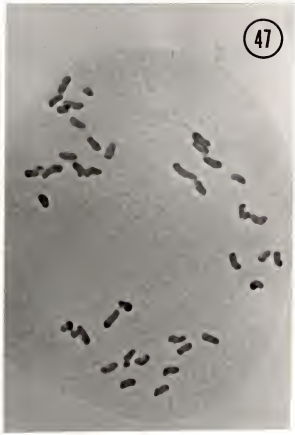
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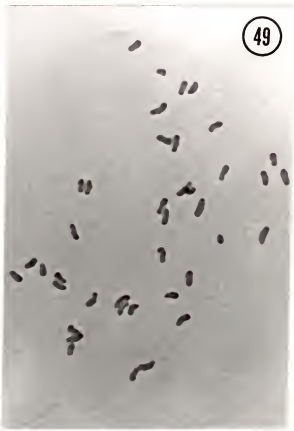
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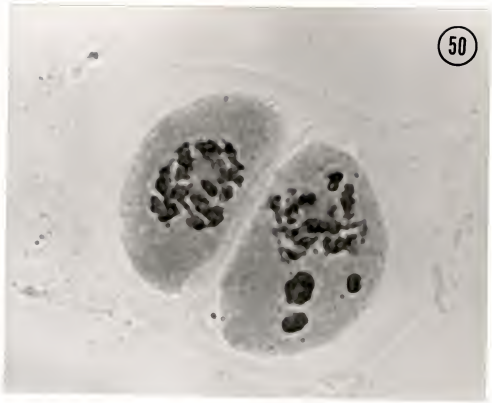
Figures 50, 51. Telophase I and II stages showing the unequal distribution of chromatin materials in pollen mother cells of nullisomic plants in the monosomic line 213-3-1. 50. Telophase I. 51. Telophase II.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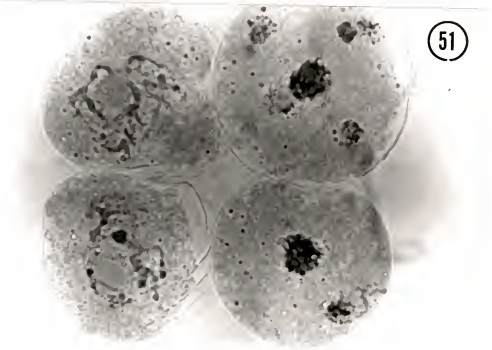
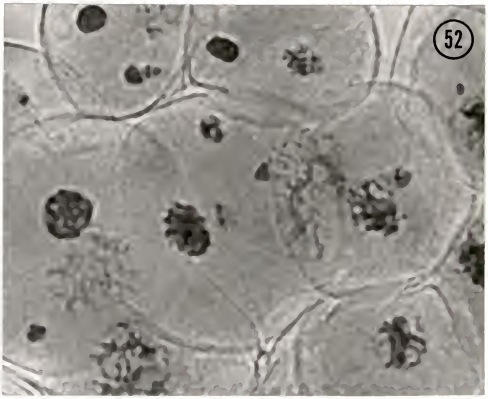

51

Figure 52. Telophase II, showing unequal distribution of chromatin materials in microspores of a nullisomic plant in the monosomic line 213-3-1.

Figure 53. Sterile pollen grains of a nullisomic plant in the monosomic line 213-3-1.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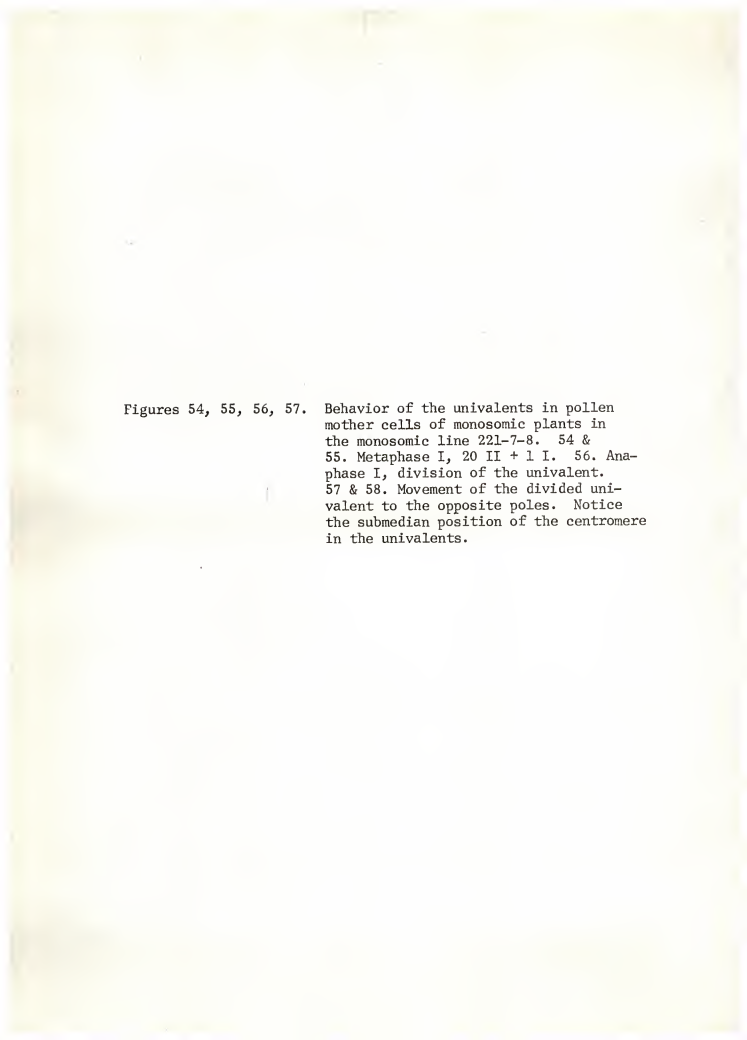
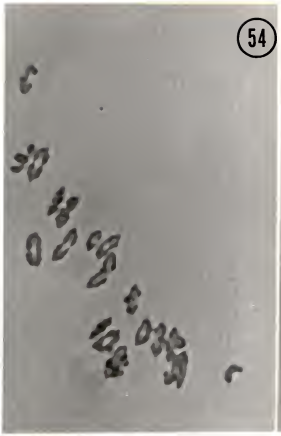


Figures 54, 55, 56, 57. Behavior of the univalents in pollen mother cells of monosomic plants in the monosomic line 22l-7-8. 54 & 55. Metaphase I, 20 II + l I. 56. Anaphase I, division of the univalent. 57 & 58. Movement of the divided univalent to the opposite poles. Notice the submedian position of the centromere in the univalents.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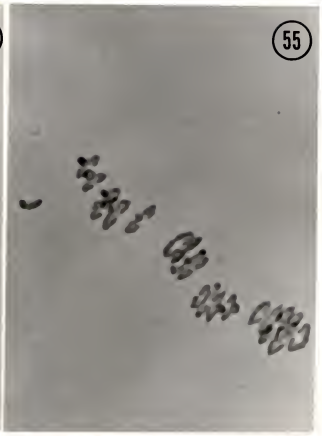
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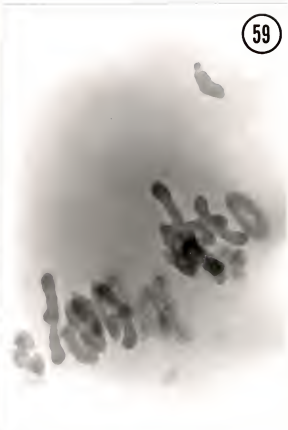
Figure 58. Movement of the divided univalent to the opposite poles in pollen mother cell of a monosomic plant in the monosomic line 213-3-1.

Figure 59. Metaphase I with 20 II + 1 I, in a pollen mother cell of a monosomic plant in the monosomic line 221-7-8. Notice a constriction at the end of the long arm of the univalent.

Figure 60. Normal pollen grains of a monosomic plant in the monosomic line 221-7-8.


58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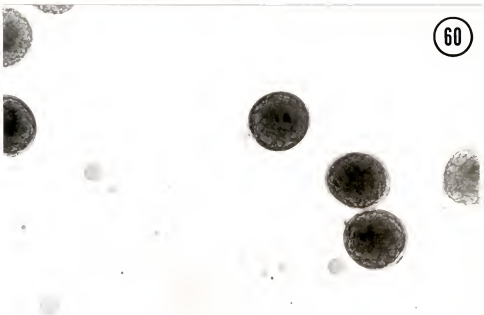
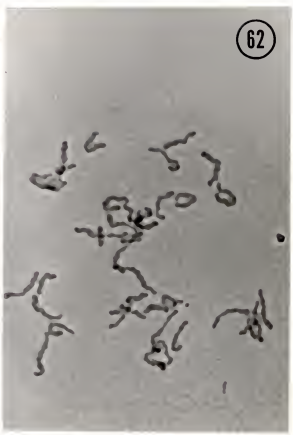

60

Figures 61, 62, 63, 64. Early prophase I stages showing 20 loosely associated homologous pairs in the pollen mother cells of nullisomic plants in the monosomic line 221-7-8. Notice the thin long thread like structure associated with the chromosomes in Figure 64, the nature of which is not clear.


61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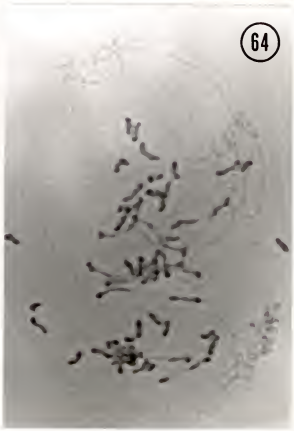
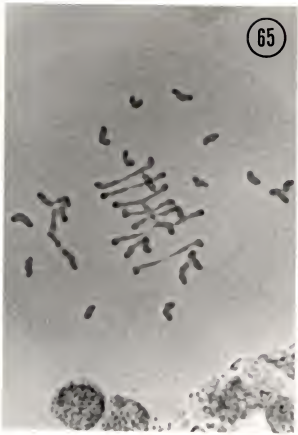

64

Figures 65, 66, 67. Late metaphase I stages showing desynapsis of certain pairs of chromosomes resulting in univalents in pollen mother cells of nullisomic plants in the monosomic line 22l-7-8.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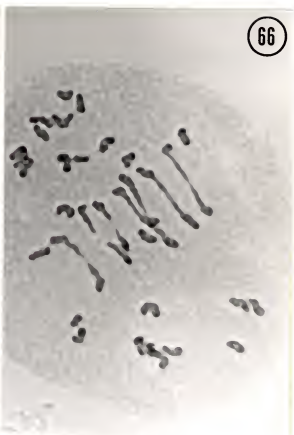
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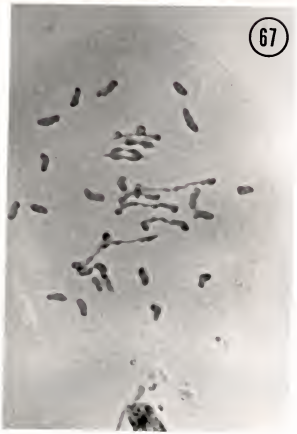
67

Figures 68, 69, 70. Anaphase I stages showing the movements of 14 chromosomes, in groups of 7 each, to the opposite poles leaving 26 univalents in the middle of the pollen mother cells in nullisomic plants of the monosomic line 221-7-8.

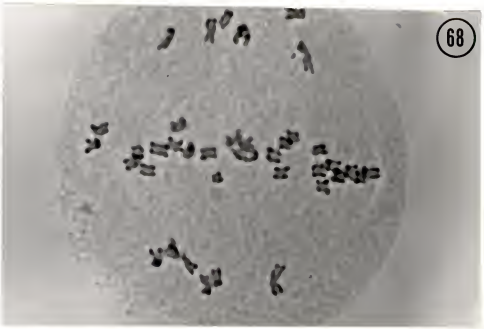
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Figure 71. Late anaphase I, showing extremely irregular division in a pollen mother cell of a nullisomic plant in the monosomic line 221-7-8.

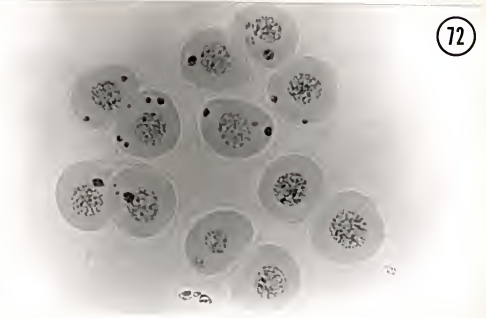


Figure 72. Telophase II, showing tetrads with varying number of micronuclei in pollen mother cells of a nullisomic plant in the monosomic line 221-7-8.

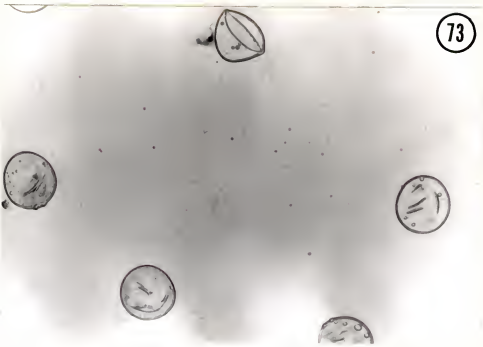


Figure 73. Sterile pollen grains of a nullisomic plant in the monosomic line 221-7-8.

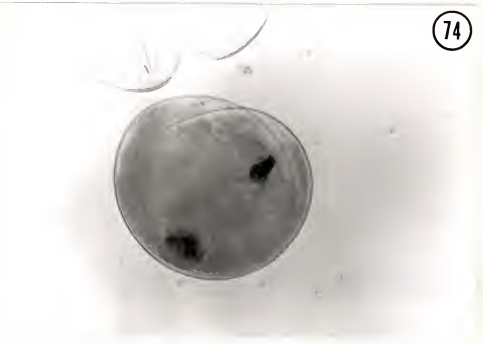
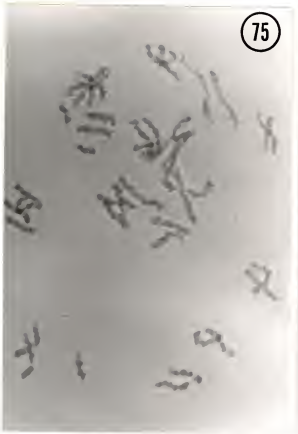


Figure 74. Sterile and giant pollen grains of a nullisomic plant in the monosomic line 221-7-8.

Figures 75, 76, 77. Chromosome pairing in pollen mother cells of a plant monotelocentric for the long arms of the critical chromosome in the monosomic line 221-7-8. 75. Early prophase I, showing 20 loosely associated homologous pairs plus telocentric for the long arm (arrow). 76 & 77. Late metaphase I stages showing desynapsis of certain pairs of chromosomes resulting in univalents.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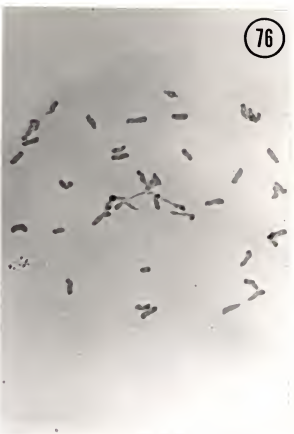
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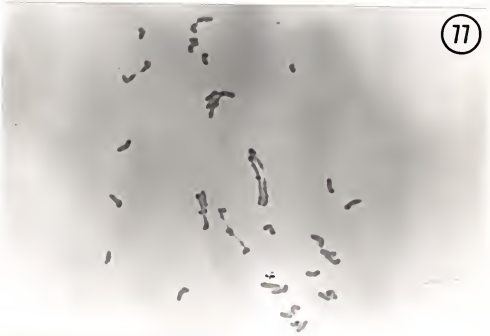
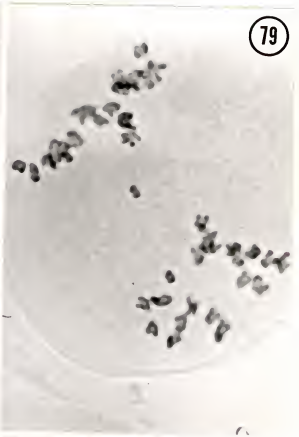

77

Figures 78, 79, 80. Chromosome pairing in pollen mother cells of plants monotelocentric for the short arms of the critical chromosome in the monosomic line 221-7-8. 78. Metaphase I, showing 20 II + a telocentric for the short arm off the metaphase plate. Notice the perfect pairing of homologous chromosomes. 79. Anaphase I, showing the division of the telocentric short arm. 80. Anaphase I, showing the movement of the divided telocentric short arm to the opposite poles.


78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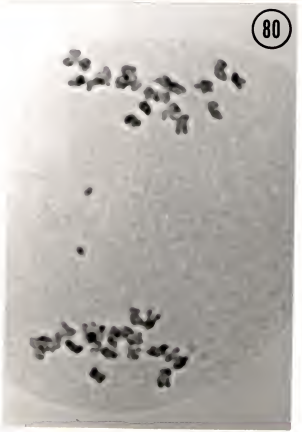
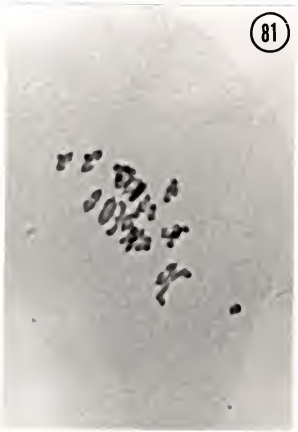

80

Figures 81, 82, 83, 84. Chromosome pairing in pollen mother cells of a plant monoisosomic for the short arm of the critical chromosome in a monosomic line 221-7-8. 81. Metaphase I, showing 20 II + a isochromosome off the plate. 82. Anaphase I, division of the isochromosome. 83. Anaphase I, showing the misdivision of the isochromosome. 84. Anaphase I, showing the movement of the divided isochromosomes to opposite poles.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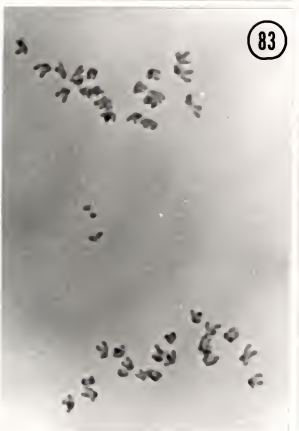
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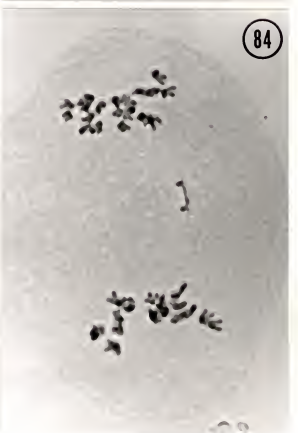
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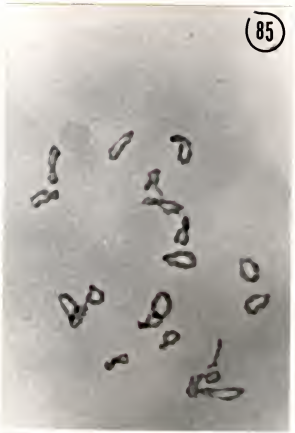
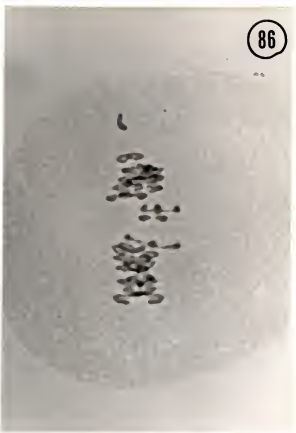
Figures 85, 86, 87, 88. Behavior of univalents in pollen mother cells of monosomic plants in the monosomic line 473-6-6. 85. Diakinesis, 20 II + 1 I. 86. Metaphase I, 20 II + 1 I. Univalent off the metaphase plate. 87. Anaphase I, division of the univalent. 88. Telophase I, movement of the divided univalent to the opposite poles. Notice the subterminal position of the centromere in the univalents.


85

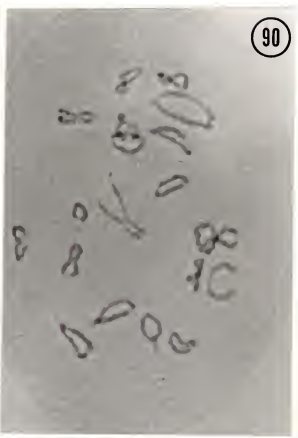

86


87


88

Figures 89, 90, 91, 92. Chromosome pairing in pollen mother cells of nullisomic plants in the monosomic line 473-6-6. 89 & 90. Diakinesis, 20 II. 91 & 92. Metaphases I, 20 II.


89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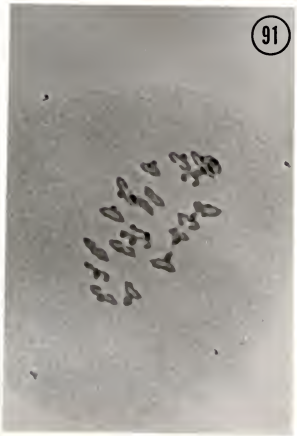
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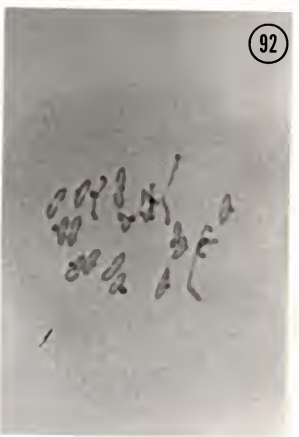
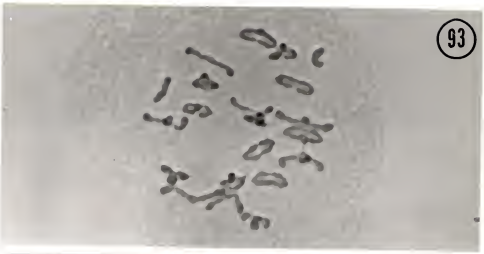

92

Figure 93. Metaphase I, showing 19 II plus a desynapsed pair as two univalents in pollen mother cell in a nullisomic plant of monosomic line 473-6-6.

Figure 94. Normal microspores formation at the telophase II in nullisomic plants of monosomic line 473-6-6.

Figure 95. Abnormally long and thin chromosomes at late metaphase I in very few pollen mother cells in nullisomic plants of the monosomic line 473-6-6.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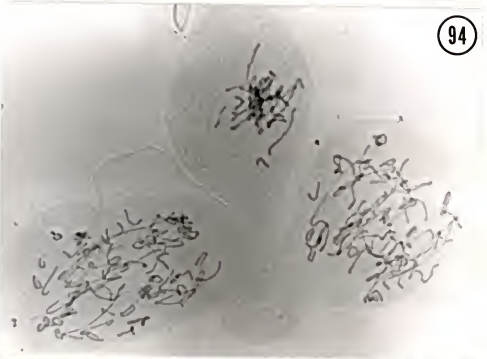

94

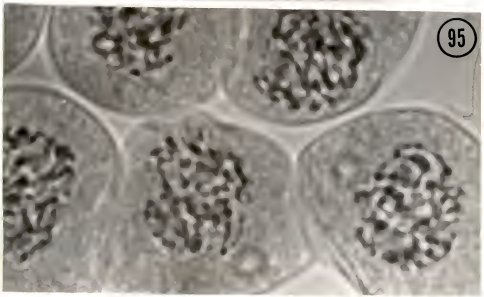
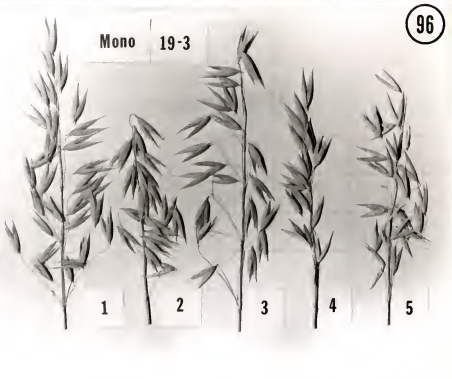
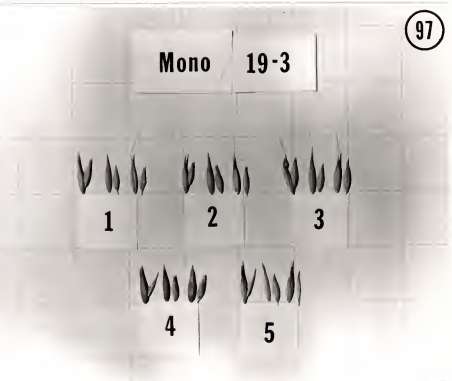

95

Figure 96. Panicles from derivatives of a selfed monosomic plant in the monosomic line 19-3-1. (1) Disome. (2) 20 II + 1 heteromorphic bivalent. (3) Monosome. (4) 20 II + 2 isochromosomes for the short arm + 1 telocentric for the long arm. (5) Monotelocentric for the long arm.

Figure 97. Kernels of the types described in Figure 96 in the same sequence.


96
Mono 19-3
1
2
3
4
5


97
Mono 19-3
1
2
3
4
5

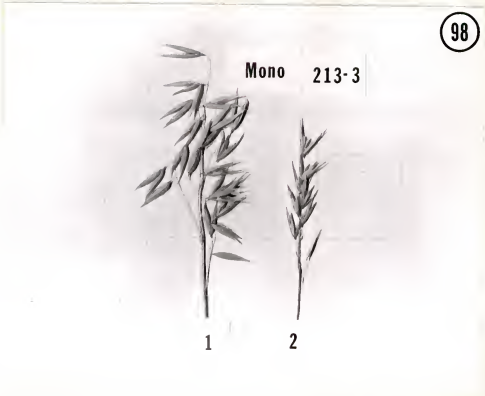


Figure 98. Panicles of monosomic and nullisomic plants in the monosomic line 213-3-1. (1) Monosome. (2) Nullisome.

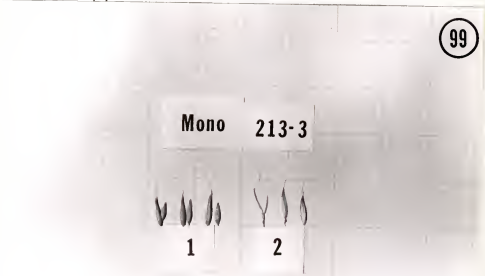
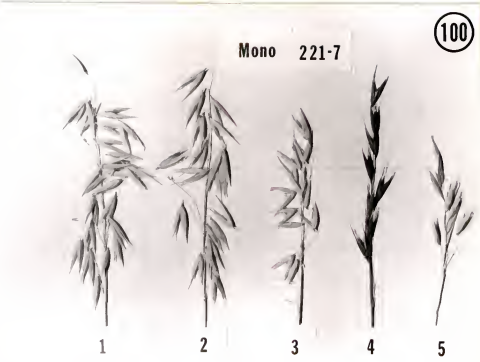


Figure 99. Kernels of the monosomic and nullisomic plants in the monosomic line 213-3-1. (1) Monosome. (2) Nullisome.

Figure 100. Panicles of derivatives of a selfed monosomic plant in the monosomic line 221-7-8. (1) Disome. (2) Monosome. (3) Monotelocentric for the long arm. Notice the normal grain characters. (4) Nullisomic. Notice the homozygous fatuoid characters. (5) Monotelocentric for the short arm. Notice the homozygous fatuoid characters.

Figure 101. Kernels of the types described in Figure 100 in the same sequence.


100
Mono 221-7
1
2
3
4
5


101
Mono 221-7
1
2
3
4
5

Figure 102. Comparative view of rachis base of disomic and nullisomic plants in the monosomic line 221-7-8. Notice the crooked rachis base of the nullisome.

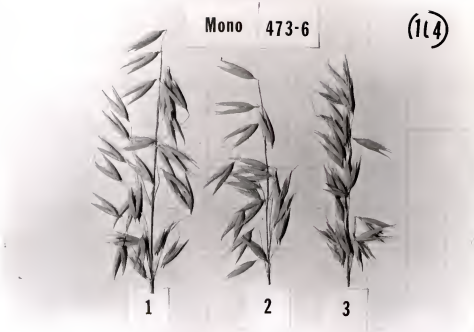
Figure 103. Comparative view of leaves in disomic and nullisomic plants in the monosomic line 221-7-8. Notice the yellowish spots in the nullisome.

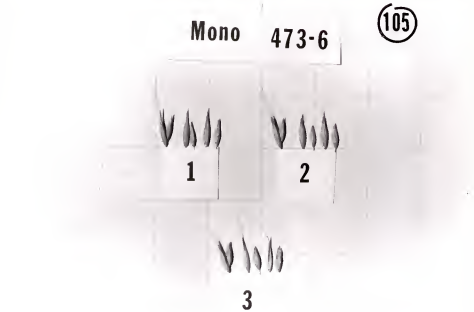

DISOMIC
NULLI


DISOMIC
NULLI

Figure 104. Panicles of disomic, monosomic, and nullisomic plants in the monosomic line 473-6-6. (1) Disome. (2) Monosome. (3) Nullisome.

Figure 105. Kernels of disomic, monosomic, and nullisomic plants of the monosomic line 473-6-6. (1) Disome. (2) Monosome. (3) Nullisome.


Mono 473-6
(104)
1
2
3


Mono 473-6
(105)
1
2
3

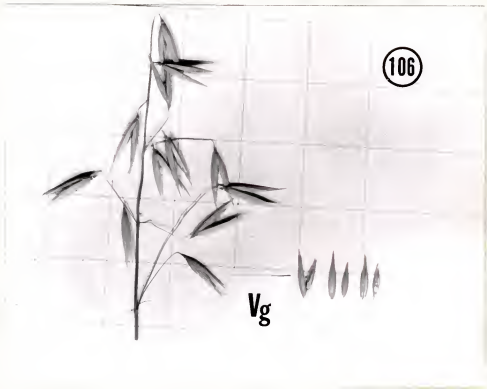


Figure 106. Panicle and kernels of Victorgrain.

DISCUSSION

Artificial induction of aneuploids using ionizing radiations and mutagenic chemicals seems to offer bright prospects for developing monosomic lines for each chromosome in hexaploid oats. From 84 lines, cytologically examined from different treatments, eight monosomic lines were extracted, resulting in a frequency of 9.52 per cent -- a figure well over that of spontaneously-occurring monosomics in hexaploid oats (20, 43).

Costa-Rodrigues (12), the first to use X-rays for induction of monosomics in hexaploid oats, A. sativa, obtained results of 7.2 per cent monosomic plants. He pointed out that most of his monosomes involved smaller chromosomes and suggested that only the smaller chromosomes can be eliminated with X-rays. However, in the present investigation, two of the monosomes involved the longest median chromosome of the A genome. Thus it is clear that any of the chromosomes can be eliminated with ionizing radiations. Sparrow et al. (82) reported that the larger the chromosome size, the greater the number of breaks produced per unit dose, or conversely, the smaller the chromosome size, the higher the dose required to produce one break. In the light of the above findings, it is expected that the higher number of breaks should be produced in the larger chromosomes, and that consequently monosomes may involve such chromosomes more frequently.

Detailed cytomorphological investigations of five monosomes, namely, 19-3-1, 136-2-1, 213-3-1, 221-7-8, and 473-6-6, chosen out of eight monosomes in the present investigation revealed that all except monosome 136-2-1 involved different chromosomes. Monosome 136-2-1 was the same as 19-3-1 and monosome 221-7-8 was analogous to the monosome-c reported by Nishiyama (51, 52). The frequencies of disomics, monosomics, and nullisomics of selfed monosomic plants varied from 0-3.03, 38.71-85.10, and 0-61.29 per cent, respectively, in different monosomic lines. The frequencies of monotelosomics together with monoisosomics were found to be 9.70, 0, 9.11, and 17.07 per cent for monosomes 19-3-1, 213-3-1, 221-7-8, and 473-6-6, respectively. Plants with 20 II + 1 heteromorphic bivalent constituted a frequency of 3.25 per cent in monosome 19-3-1.

These marked variations in the frequencies of disomics, monosomics, nullisomics, and other chromosomal types in the progenies of selfed monosomic plants in different monosomic lines, are attributed to variation in the frequency of functioning 20 chromosome pollen grains. However, there seems to be a certain kind of selection for 20 chromosome eggs in monosomes 19-3-1 and 213-3-1. The absence of nullisomics and the very high frequency (85.10%) of monosomics in monosome 19-3-1 indicates inviability of deficient pollen grains and viability of deficient eggs. On the other hand, high frequencies of nullisomics in monosomes 213-3-1 (61.20%) and 221-7-8 (39.39%) indicate that both deficient pollen grains and eggs performed equally well.

Nishiyama (51), Philp (58, 59), McGinnis and Taylor (44), and Lafever and Patterson (35) reported a close agreement between frequencies which were expected (calculated from micronuclei counts) and

observed (based on actual chromosome counts of each plant in progenies of selfed monosomics) in disomics, monosomics, and nullisomics in different monosomic lines. No such relationship was found for any of the four monosomic lines in the present investigation. This finding is supported by the recent reports of Hacker (19) and Schulenburg (72). Hacker (19) feels that frequencies of disomics, monosomics, and nullisomics are determined largely by differential certation of pollen deficient for different chromosomes, rather than by differences in the inclusion of chromatids of unpaired chromosomes in tetrad nuclei.

It is interesting to note that Nishiyama (51) got a close agreement between expected and observed frequencies only after readjustment for observed nullisomic plants. The basis for readjustment was based on the assumption that observed sterility of 57.24 per cent on monosomic plants was caused by the death of 40 chromosome zygotes. Surprisingly enough, in the present investigations the sterility of monosomic plants in monosome 22l-7-8 was found to be only 9.24 per cent. Thus it is very likely that sterility of monosomic plants reported by Nishiyama (51) may have been due to species differences or environmental influences and had nothing to do with nullisomic zygote inviability. Furthermore, if nullisomic zygote inviability occurs, it is hard to explain how and why a good percentage of zygotes survived to maturity.

Germination percentages in progenies of different selfed monosomic lines were quite high, indicating no selection against nullisomic plants. Chang and Sadanaga (7) have reported high germination percentage for their five monosomic lines which were different from those reported here. Morphological markers, along with frequencies of

disomics, monosomics, and nullisomics, can be effectively used to distinguish one monosome from others. Two of the monosomic lines 213-3-1 and 221-7-8 were clearly distinguished by narrow leaves and fatuoid characters, crooked rachis bases, and yellowish leaf spots, respectively. Because of the distinct frequencies of disomic, monosomic, and nullisomic plants in progenies of selfed monosomes in different lines, they were easily distinguishable from each other.

Great reduction in fertility of monosomic plants as compared to disomics has been reported by a number of workers (7, 16, 42, 72) in A. sativa. In the present investigation however, fertility of monosomic plants in different lines varied from 82.90 to 90.76 per cent as compared to 92.55 to 100 per cent for disomic plants. The fertility of disomic plants may be a little higher because of the limited number of plants.

Nullisomic plants in different monosomic lines showed much reduction and variation in their fertilities. No nullisomic plants were obtained in monosomic line 19-3-1, whereas nullisomic plants in monosomic lines 213-3-1, 221-7-8, and 473-6-6 showed mean fertilities of 0, 3.5, and 36.91 per cent, respectively. The decreased fertilities of nullisomic plants in monosomic lines 213-3-1 and 221-7-8 is attributed to pairing failure, and of 473-6-6 because of inherent low fertilities of nullisomics. Nishiyama (51) reported complete sterility for monosome-c (here 221-7-8) and attributed it to pairing failure. Ramage and Suneson (68) reported a nullisomic plant with 10 per cent seed set in A. byzantina; Hacker and Riley (21) reported few fertile nullisomics without any actual data; and Lafever and Patterson (35) reported a nullisomic plant with 0 to 55.0 per cent fertility, all in

A. sativa. Lafever and Patterson (35) conducted the experiments under controlled conditions and reported cooler temperature increased seed set. In most cases where sterile nullisomics have been reported, it is not clear whether sterility was due to genic, chromosomal, or environmental factors. If the environmental factors are the cause, cytogenetic studies of nullisomics need to be conducted in controlled environments.

Data on mean plant heights showed no appreciable differences between disomics and monosomics, but nullisomics did show marked reduction in mean plant height. Also, mean plant height of monosomic plants in monosomic lines 213-3-1 and 221-7-8 were shorter than monosomes 19-3-1 and 473-6-6. A plant with 20 II + 2 isochromosomes + a telocentric for the long arm in monosomic line 19-3-1 showed great reduction in plant height. These findings can be explained on the basis that different chromosomes have differential effects because of their deficiency or duplication elsewhere in the genomes.

Genome relationships in Avena have been a controversial subject of investigations and have still not been clarified. Based on pairing studies in hybrids between different species of Avena, Nishiyama (50, 53, 54) designated the genomes of A. strigosa as A, of A. barbata as AB', and A. fatua as ABC. He pointed out that there was some homology between the B' genome of tetraploid and B genome of hexaploid species. From the studies of Rajhathy (63), Rajhathy and Morrison (66), Rajhathy and Dyck (65), and Thomas and Jones (83), it is evident that hexaploid species of oats have A, C, and D genomes and A genome is present in its original or modified form in all the species, whereas genomes C and D have not been found so far in any diploid and tetraploid species

studied. Of course, these conclusions are based on observed pairing between species hybrids, assuming that the pairing was the direct result of homology of the chromosomes and not due to any other factors. This assumption is questionable, since pairing has been shown to be under genic controls.

Karyotype analysis of hexaploid oats by Rajhathy and Morrison (66), Rajhathy (64), and Gill et al. (17) have greatly helped in the identification of chromosomes otherwise hard to distinguish. In the present investigation the system of karyotype analysis proposed by Rajhathy (64) was adapted because of its definite merits. However, his standard karyotype of A. sativa was not used because the author feels that it would be appropriate only after genetic activities of all the chromosomes in both of the species are clearly understood and the genome relationship worked out.

Three pairs of satellited chromosomes were recognized, as compared to two pairs reported by Gill et al. (17). Rajhathy (64) reported the three pairs of satellited pairs of chromosomes in A. sativa. From karyotype analysis the missing chromosomes in monosomes 19-3-1, 213-3-1, and 473-6-6 were identified as M-3, ST-7, and ST-17, respectively. No serious efforts have been made by previous workers to identify the missing chromosome based on karyotype analysis. In good preparations of somatic metaphase 11 to 14 pairs of chromosomes can be identified with efforts. The identification of others is more difficult. A good somatic metaphase was not obtained for monosome 221-7-8, the reasons for which are not clear. From meiotic studies, the missing chromosome in the above line was found to be submedian; it may be either SM-12 or 13.

Percentage univalent transmission rate from the male side as determined by micronuclei counts at tetrad stage was found to be 19.07, 11.65, 9.32, and 21.37 per cent for monosomes 19-3-1, 213-3-1, 221-7-8, and 473-6-6, respectively. Nishiyama (51) reported univalent transmission rate for monosome-c to be 14.28 per cent; Philp (58, 59) for monosome V, 7.0 and monosome L, 6.0 per cent; McGinnis and Taylor (44) for monosome involved in chlorophyll production, 16.8 per cent; and Lafever and Patterson (35) for monosome involved in sterility, 6.03 per cent. From these transmission rates these workers calculated the expected frequencies of disomics, monosomics, and nullisomics, which agreed well with the observed frequencies. In a few recent experiments (19, 72) including this one, no such agreement was found between observed and expected frequencies. It is interesting to note that although observed frequencies of disomics, monosomics, and nullisomics in monosome 221-7-8 reported here, and monosome-c reported by Nishiyama (51), agreed very well, univalent transmission rates based on micronuclei counts were different. Because of the errors associated with the scoring of the univalent transmission rates (personal as well as environmental) and because of certain erroneous assumptions (i.e., the same transmission rate on both male and female sides and no selection against 20 chromosome gametes), the usefulness of this method in predicting the frequencies does not seem to be reliable.

Meiotic behavior of nullisomics in two of the monosomic lines, namely 213-3-1 and 221-7-8, was found to express abnormal pairing of the homologous chromosomes, as presented in "Experimental Results." Those results are discussed here along with findings of some other workers. Thus nullisomic plants in monosome 213-3-1 showed complete

pairing failure, and 28 chromosomes moved in groups of 14 each to opposite poles, leaving 12 univalents in the middle of the pollen mother cells (PMCs). However, nullisomic plants in monosome 221-7-8 showed 20 loosely associated pairs in early prophase, but by late diakinesis or early metaphase I, all the paired chromosomes except 0-10 II formed univalents. The movement of the chromosomes was just opposite to that described for nullisome 213-3-1. Thus 14 chromosomes in groups of 7 each moved to opposite poles, leaving 26 univalents in the middle of the pollen mother cells (PMCs).

Since pairing, along with chromosome duplication, is supposed to be responsible for chromosome movement at anaphase I, the validity of the statement regarding the movements of chromosomes in nullisomic plants of monosomic line 213-3-1 can be questioned. As far as observed, no pairing was detected, but prophase stages earlier than these studied here should be checked for any pairing. Further discussion of this phenomenon is limited because of the lack of information about chromosome pairing and their movements at any level.

It is postulated that the missing homologous pair in nullisome 213-3-1 is from the A genome of A. byzantina and controls the initial pairing of all the chromosomes, but more specifically the pairing of 14 chromosomes of the A genome. The missing pair from nullisome 221-7-8 controls the steps in the pairing process of 28 chromosomes and in its absence desynapsis of 13 pairs of chromosomes results in 26 univalents. However, for normal and perfect pairing, both homologous pairs or even one chromosome from each pair, are necessary.

Meiotic behavior of nullisomic plants in monosome-c (analogous to 221-7-8) was first reported by Huskins (24, 25) and then by Nishiyama (51, 52). These workers reported extreme irregularity in meiotic divisions resulting in complete sterility of nullisomic plants. With the use of monotelocentric plants for long and short arms Nishiyama (51, 52) located the pairing genes on the long arm and genes for inhibition of fatuoid characters on the short arm of the c-chromosome. Huskins et al. (27) reported the opposite results.

Presence of monotelocentric plants both for long and short arm in line 221-7-8 led to investigation of the pairing of homologous chromosomes in these plants. It was found that monotelocentric plants for the long arm showed very irregular pairing like that described for nullisomic plants, whereas plants monotelocentric and monoisosomic for the short arms showed normal pairing like monosomic or disomic plants, with 20 II + a telocentric fragment. In morphological appearance plants monotelocentric for the long arm showed normal grain characters, and plants monotelocentric or monoisosomic for the short arms showed homozygous fatuoid characteristics like nullisomic plants.

Thus in monosome 221-7-8, the genes for pairing were located on the short arm of the missing submedian chromosome and genes for inhibition of fatuoid characters on its long arm. No monotelocentric plants were obtained in monosome 213-3-1, so it was not possible to locate the pairing genes on the specific arms. Since loss of homologous pairing in nullisome 213-3-1 results in complete pairing failure and in 221-7-8 separation of paired chromosomes during advanced prophase and metaphase I stages, the terms asynapsis and desynapsis, respectively, have been used for the two lines.

Since the recent report of Nishiyama and Tabata (56) that the hexaploid oats have physically or functionally lost the synaptic factors in the A and B genomes and are dependent for their normal chromosome pairing upon the synaptic factor in the C genome, three different chromosomes, two satellited of the A genome by Hacker and Riley (21), and one subterminal in the B or C genomes by Schulenburg (72), have been reported to control the normal pairing of homologous chromosomes in A. sativa. If a similar situation exists in A. byzantina, pairing genes, besides the two reported here, will no doubt be discovered.

Based on the results presented in the "Experimental Results" a number of morphological characters are associated with critical chromosomes in different monosomic lines. Thus from fertility data of plants with monotelocentric for the long and the short arms and with 20 II + 2 isochromosomes for the short arms + a telocentric for the long arm in monosome 19-3-1, it is concluded that the short arm carries the gene or genes responsible for fertility. Furthermore, the plant with the latter chromosomal constitution produced seeds with dark gray lemma color. Thus the short arm carries the gene or genes for gray color production, the expression of which is inhibited by factors on the other chromosomes and produces normal grain color, whereas an excessive dose of this gene results in dark gray color. Since fatuoid seeds produced on nullisomics, and on plants with monotelocentric for the short arm, also have dark gray lemma, it is possible that there may be factors present on the long arm of the critical chromosome in monosome 22l-7-8 for the suppression of gray factors present on the short arm of the critical chromosome in monosome 19-3-1.

The characteristic of narrow leaf was found to be associated with the missing pair of chromosomes in monosome 213-3-1. Disomic and monosomic plants had normal leaves. Philp (59) associated narrow leaf characteristic with a different chromosome in A. sativa.

The long arm of the critical chromosome in monosomic 221-7-8 carried genes for suppression of fatuoid characteristic, normal rachis base and normal leaf color, and its absence resulted in fatuoid characters, crooked rachis bases, and yellowish leaf spots. Gauthier and McGinnis (16) associated abnormal rachis base (i.e., "kinky neck") with chromosome 20 of A. sativa, which is different from the submedian chromosome (SM-13 or 14), the absence of which causes crooked rachis base like chromosome 20.

The discrepancies in association of narrow leaf and abnormal rachis base, with different chromosomes in the two species A. sativa and A. byzantina, may be due to their evolutionary differentiations. It is interesting to note that Hacker and Riley (21) have observed similar inconsistencies with regards to other characters, even in different varieties of A. sativa.

Since nullisomic plants in monosomic 473-6-6 were two weeks later in maturity than the disomics or monosomics and were dark green in the floret base, factors for early maturity and normal floret base color are associated with missing chromosomes.

F_2 segregation of seeds from crosses between Victorgrain and different monosomic lines, tested in H. victoriae toxin, did not give expected frequencies of resistant plants, i.e., equivalent to frequencies of nullisomic plants in different monosomic lines, and thus the latter did not involve the chromosome carrying the vb locus.

SUMMARY

Out of eight lines screened from X-irradiated and chemically treated populations of the oat variety Victorgrain Avena byzantina C. Koch, five monosomic lines, designated as 19-3-1, 136-2-1, 213-3-1, 221-7-8, and 473-6-6, were studied from years 1963 to 1965.

All the five monosomic lines involved different chromosomes except 136-2-1, which was the same as monosome 19-3-1. In the progenies of selfed monosomes, the frequencies of disomics varied from 0 to 3.03 per cent; monosomics, from 38.71 to 85.10 per cent; nullisomics, from 0 to 61.29 per cent; and other chromosomal types (monotelocentrics, monoisosomics, and with 20 II + 1 heteromorphic bivalent) from 0 to 17.07 per cent. Germination percentage for all the monosomics was high and ranged from 84.0 to 99.0 per cent.

No agreement was found between the frequencies which was observed (based on the determination of the chromosome number of each plant in progenies of selfed monosomics) and those which were expected (calculated from micronuclei counts at tetrad stage of monosomic plants in different monosomic lines) among disomic, monosomic, and nullisomic plants in any of the monosomic lines.

In the progenies of selfed monosomes the fertilities of disomics ranged from 92.55 to 100 per cent; monosomes, from 82.90 to 90.76 per cent; and nullisomics, from 0 to 36.91 per cent. Nullisomic plants in all the monosomic lines showed great reduction in plant height

over their disomics or monosomics. Overall reduction in plant height also occurred in monosome 213-3-1 and 221-7-8 as compared to monosomes 19-3-1 and 473-6-6.

Karyotype analyses made of the critical chromosomes in monosomes 19-3-1, 213-3-1, and 473-6-6 were designated as M-3, ST-7, and ST-17, respectively. From meiotic anaphase studies the missing chromosome in the monosome 221-7-8 was found to be a submedian which could be either SM-13 or 14.

Meiotic behavior in disomics, monosomics in all the lines, and of nullisomics in the monosome 473-6-6, were found to be normal. Disomics formed 21 II monosomes 20 II + 1 I and nullisomics 20 II at diakinesis and metaphase I stages. The univalent transmission rates in different monosomic lines varied from 9.32 to 21.37 per cent.

Nullisomic plants in monosomes 213-3-1 and 221-7-8 showed asynapsis and desynapsis, respectively. Absence of the critical pair in nullisomics of monosome 213-3-1 caused the pairing failure of all the chromosomes in the early prophase and influenced the movement of chromosomes at anaphase I. Thus at anaphase I, 28 chromosomes in groups of 14 each, moved to opposite poles, leaving 12 univalents in the middle of the pollen mother cell (PMC), whereas in nullisomics of monosome 221-7-8, loose chromosome pairs were observed in the early prophase, but by late prophase or metaphase I only 0 to 0-10 II were left and the rest of the pairs formed univalents. The movement of the chromosomes was opposite to that described for nullisomic 213-3-1. Thus 14 chromosomes in groups of 7 each moved to opposite poles, leaving 26 univalents in the middle of the pollen mother cell (PMC).

From these observations it is suggested that the critical chromosome in monosome 213-3-1 is from the A genome. It controls the pairing of all the chromosomes, but more specifically of 14 chromosomes from the A genome, whereas critical chromosome in monosome 221-7-8 controls more specifically the later steps in the pairing process of 28 chromosomes from the C and D genomes.

From morphological and cytogenetical studies genes for gray seed color production and fertility were placed on the short arm of M-3, genes controlling normal pairing and normal leaf width placed on ST-7, another gene for normal pairing on the short arm, and genes for inhibition of fatuoid characters, crooked rachis bases and yellowish leaf spots placed on the long arm of the critical chromosome in monosome 221-7-8, and genes for normal maturity and floret base color placed on ST-17 in monosome 473-6-6.

The gene vb controlling the resistance to fungus Helminthosporium victoriae M. and M. was not associated with the critical chromosomes in any of the monosomic lines tested.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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